Reed College Biosafety Manual

June 2023

0.1 Exposure Incident

Report exposure immediately; you may need immediate therapy.

Needlesticks/puncture wounds:

- Wash the affected area with antiseptic soap and warm water for 15 minutes

Mucous membrane exposure:

- Flush the affected area for 15 minutes using an eyewash.

For all exposure incidents:

1. Notify Principal Investigator, manager or supervisor (if available) to initiate accident or exposure incident report.
2. If you are experiencing an emergency, **call 911.**
    - Otherwise, have your supervisor call **(503) 216-7960** between 8 am and 5 am, Monday through Friday, to make an appointment at:

        Providence Occupational Health - Clackamas
        9290 SE Sunnybrook Blvd, Suite 210
        Clackamas

    - If no appointments are available, go to:

        Kaiser Permanente
        503/652-2880
        10180 SE Sunnyside Rd
        Clackamas

Supervisors, call Broadway Cab at 503/333-3333 to provide transport in non-emergency situations. Tell the cab company your name, department, and Reed College account (contact Community Safety for the number). Please consider accompanying your employee.

0.2 Foreword

This manual provides a core set of biosafety practices and procedures for the safe handling of known biohazards and potentially infectious materials.

The manual focuses on Biosafety Levels 1 and 2, as all laboratories at Reed College fall within these designations. No work with Biosafety Level 3 agents may be conducted at Reed College without prior approval.

Registration and training information are provided along with details on work practices, safety equipment and facility design. It is the responsibility of the Principal Investigator or Supervisor to ensure that his/her laboratory is in compliance. That responsibility includes identification of the risk or hazards associated with their research and the application of the appropriate safety procedures. Please read the section on responsibilities for additional information.

We urge you to use the manual as a road map to compliance within your laboratory. Consult the sections relevant to your research and apply the appropriate safety procedures. The Environmental Health and Safety Office is available for consultation if you have any questions or concerns with any aspect of the Biosafety Program at the College.

1.0 Introduction

1.1 Emergency Phone Numbers and Office of Environmental Health and Safety Contacts

Ambulance/Fire/Police 911

Biological/Chemical/Radiological Emergencies 777-7788

- (Monday – Friday, 8:30 AM to 5:00 PM, at other times call Community Safety at 503/788-6666)

1.2 Responsibilities

1.2.1 Principal Investigator

The Principal Investigator has the responsibility and authority for assessing risks, establishing policies and procedures, training personnel and maintaining the facility and equipment.

The Principal Investigator is responsible for:

- Performing appropriate risk assessment of research projects.
    - The level of detail should be dependent on the hazard associated with the organism under study (e.g., an assessment of risk associated with research on BL2 agents might reasonably be less detailed than a risk assessment of unknown agents).
    - Each evaluation should be completed before work is undertaken and the project should be reassessed periodically as new data is obtained.
    - The assessment should include an analysis of the risks posed by the particular organism under investigation and of any specific research methods that may affect that risk (e.g., procedures requiring highly concentrated amounts of virus or inoculation of laboratory animals).
    - No human, plant, or animal pathogen should be studied without prior written approval of the Institutional Biosafety Committee.
    - The agents must be registered and information about these agents must be provided to the Office of Environmental Health and Safety. Refer to the IBC webpage (https://www.reed.edu/ibc/) for more details.
- The application of appropriate safety practices and procedures within their laboratories and instructing students and staff of potential hazards.
- Approving research personnel to work in the laboratory and documenting that personnel are competent to conduct the work.
- Developing policies governing the operation of the laboratory and implementing protocols to ensure safe operation.
- Maintaining a liaison with Reed College's EHS Office.
    - Liaison can be the Principal Investigator.

- Registering research work involving recombinant DNA with the Institutional Biosafety Committee. The Principal Investigator must complete the “Initial Classification Form” and "Recombinant DNA (rDNA) Research Questionnaire" (RDRQ) application. The application must have details of the nature of the proposed experiments and an assessment of the levels of physical and biological containment required for them as established by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines).

1.2.2 Research Personnel

Research Personnel are responsible for:

- Complete requirements for approval to work in the laboratory and ensure that all work is conducted in compliance with Reed College, NIH, CDC, OSHA and other applicable guidelines. Follow the Reed College Biosafety Manual.
- Learn the operating procedures for the laboratory, the potential hazards of the infectious agents in use and emergency procedures. Help maintain the facility in good working condition.
- Report to the Principal Investigator any medical restrictions, reportable illnesses, and any event that may be an exposure or result in the creation of a potential hazard. Report all irregular conditions.
- If inexperienced in handling human pathogens or tissue cultures, receive training and demonstrate proficiency in standard microbiological practices from the Principal Investigator.
- Complete any medical surveillance requirements.
- Perform assigned responsibilities. The operation of the facility is the responsibility of the users; therefore a number of tasks must be assigned. These tasks are as follows:
- Training
- Autoclaves and waste
- Freezers
- Cleaning
- Vacuum trap and filter maintenance
- Maintenance of supplies, including personal protective equipment
- Security of infectious agents; i.e. store infectious agents in a locked freezer in a locked laboratory

1.2.3 Office of Environmental Health and Safety

The Office of Environmental Health and Safety:

- Provides consultation on operation of the laboratory to ensure compliance with CDC, NIH, OSHA and state criteria.
- Provides information on regulations that apply to the laboratory.
- Advises on safe methods for new procedures, and provides advice in the event of large or high hazard biohazardous material spills.

- At a minimum, inspect laboratory spaces on a yearly basis to ensure continued compliance with safety regulations.

The Biosafety Officer (BSO) is responsible for the implementation of policy guidelines recommended by the Institutional Biosafety Committee (IBC). The BSO identifies potential problem areas and suggests to the IBC safety objectives to be achieved. In addition, the BSO is also the institutional Biosafety officer for recombinant DNA research. Some of the specific Biosafety services provided by the Office of Environmental Health and Safety's BSO include:

- Evaluation and inspection of laboratory facilities for work with infectious agents and other hazardous biological agents;
- Investigation of laboratory accidents;
- Periodic updates of rDNA experiments to ensure compliance with the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines);
- Maintenance of training records for compliance with federal, state and College requirements;
- Consultation to members of the Reed community in matters related to Biosafety.
- Identification and updating of areas of known and potential biohazard at Reed College on a regular basis.
- Dissemination of information for safety in biological research through periodic newsletters, demonstrations or special training courses as necessary.

The Office of Environmental Health and Safety also provides new personnel training.

1.2.4 Reed College Institutional Biosafety Committee (IBC)

The Reed College Institutional Biosafety Committee (the Committee) shall serve as defined in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf). As such, the Committee shall review applications for research involving recombinant DNA to determine whether the facilities, procedures, and practices meet the standards required by the College and the NIH. It shall, in addition, have the responsibility to certify annually to the NIH that such facilities, procedures, and practices, and the training and expertise of personnel meet NIH standards. Meetings called for the purpose of such review and certification may be open to the public. Minutes of these meetings shall be kept and made available for public inspection.

The committee's responsibilities include:

- Registering laboratories and approving containment and procedures to be used. • Advising facility users on policies related to biohazard containment. • Updating laboratory registrations periodically.
- Determining the necessity for special medical monitoring.
- Advising Reed administration on the suspension of access privileges for staff found to be in violation of policies and procedures governing facility use.

The Committee shall advise the President, Dean of Faculty, and Director of the Office of Environmental Health and Safety (EHS) on policy matters concerned with the protection of personnel from biohazardous agents including both infectious organisms and allergens that may be present in either laboratory materials or the environment. The Committee shall also recommend guidelines relating to procedures and facilities used at the College, including such matters as safety training and health surveillance.

The Committee shall offer its counsel to all College personnel regarding matters of Biosafety. The President/Dean of Faculty may ask the Committee to inform the community about developments in the general area of Biosafety.

2.0 Biosafety Requirements

The following information describes the requirements for Reed researchers as defined by the Reed College Institutional Biosafety Committee and the Environmental Health and Safety Office. It is the responsibility of each Principal Investigator to ensure the laboratory is in compliance.

2.1 Registration for the Use of Recombinant Biological Materials

All Principal Investigators are required to complete and submit an Initial Classification Form and/or RDRQ for use of recombinant DNA. A copy of these forms are in Appendix B and C The Office of Environmental Health and Safety must maintain accurate information regarding the use of biological materials (e.g., microorganisms, cell lines, human materials, animals, and toxins) by College personnel. EHS policy requires all Principal Investigators to submit accurate information annually and when there are changes during the year regarding the addition or deletion of biological materials, addition or deletion of employees, or changes in room locations. A brief description of non-exempt and exempt recombinant DNA experiments is described in Appendix E.

2.2 Human Blood, Body Fluids, Tissue and Other Potentially Infectious Materials

The Occupational Safety and Health Administration (OSHA) created the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR Part 1910.1030 (Bloodborne Pathogens Standard) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues or certain body fluids (bloodborne pathogens.) The Bloodborne Pathogens Standard applies to all employers having employees that are “occupationally exposed” to human blood or other potentially infectious materials. An employee is considered occupationally exposed if there is “reasonably anticipated skin, eye, mucous membrane, or parenteral contact with human blood or other potentially infectious materials in the performance of an employee’s duties.” Other potentially infectious materials include:

- Human cell or tissue cultures
- Organ cultures
- Any unfixed tissue or organ, other than intact skin, from a human being (living or dead)
- Human body fluids, except urine, feces, saliva or tears unless visibly contaminated with blood
- HIV- or HBV-containing culture media or other solutions
- Blood, organs or other tissues from experimental animals infected with HIV or HBV or other bloodborne pathogens

An individual is also considered occupationally exposed even if they do not have direct contact with blood or other potentially infectious material, if the employee uses equipment that is used to process or store blood, other potentially infectious materials or bloodborne pathogens.

All occupationally exposed employees are required by OSHA to attend a Bloodborne Pathogens training session prior to beginning work and annually thereafter. There are additional requirements for research laboratories and production facilities engaged in the culture, production, concentration and manipulation of HIV and HBV.

OSHA has determined that occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens such as:

- Human Immunodeficiency virus (HIV)
- Babesia species
- Human T-lymphotropic
- Borrelia species Virus Type I
- Hepatitis B virus (HBV)
- Brucella species
- Arboviruses
- Hepatitis D virus
- Leptospira species
- Spirillum minus
- Hepatitis C virus
- Francisella species
- Creutzfeldt-Jakob virus
- Plasmodium species
- Streptobacillus moniliformis
- Colorado Tick Fever viruses
- Treponema species
- Hemorrhagic Fever viruses

Consult the Bloodborne Pathogen Program for additional information on the exposure control plan, training requirements, work practices, housekeeping, engineering controls, personal protective equipment, signs/label requirements, Hepatitis B vaccination, emergency actions, exposure incident procedures, post-exposure evaluation and follow-up, and recordkeeping. The manual is available on the EHS web site. The IBC must be notified (ibc@reed.edu) when working with bloodborne pathogens in research. Additional information may be requested from the IBC.

2.3 Animals

All research experiments involving animals must be conducted in accordance with the associated Institutional Animal Care and Use Committee (IACUC)-approved protocol. Animal

research that involves a hazard (biological, radiological, or chemical) must be reflected in the approved IACUC protocol. Contact the IACUC for additional information.

The IBC/EHS must approve work with human pathogens or recombinant DNA in animals (including transgenic animals) prior to initiation. Contact the IBC (ibc@reed.edu) to initiate the approval process.

2.4 Biosafety Cabinets (BSCs) and Other Laminar Flow Benches (LFBs)

The efficacy of BSCs and LFBs depends upon the behavior of the operator, the unit's orientation in the facility, and the movement of personnel in the laboratory.

All BSCs and LFBs at Reed must be certified at least annually.

Notify the Biosafety Officer in advance when you plan to have BSCs or LFBs moved, placed in storage, transferred to a new owner, discarded, removed from Reed or obtained from another institution or manufacturer.

Reed actively discourages the purchase and use of LFBs since air is blown across the work surface into the face and torso of the operator. The IBC and EHS recognize that clean benches do not provide personal or environmental protection from infectious or potentially infectious agents, allergens, chemicals or radioactive materials. If you are using a clean bench, contact EHS for a review of your procedures.

2.5 Training

Successful completion of a range of biosafety training programs may be required prior to the initiation of your work at Reed College. Please review the following table for information on required training.

Before initiating work involving:	You must satisfactorily complete the following training:	Training Options
Human blood, other potentially infectious materials, (including human cell lines) and bloodborne pathogens.	Bloodborne Pathogen Training: Required before initiation of work and At least annually thereafter.	EHS course or Web based training or Self-study guide
Human or animal pathogens classified at BL2	Biosafety Training	EHS course

Packaging, Shipping, Transporting, or Receiving Biohazards (Infectious agents, hazardous biological toxins, and human or animal specimens)	Certified shippers for Reed College are located in Mail Services and EHS. You may not package/transport hazardous materials without specialized training.	N/A You may not perform these duties. Please contact EHS or mail services.

3.0 Medical Surveillance Program

For a particular employee, the medical surveillance program might call for any of a number of precautionary measures, including immunizations, a periodic physical examination and collection of a serum specimen.

The purpose of the medical surveillance program is to:

- recommend appropriate medical precautions to be followed
- do periodic reassessment of employees to determine if medical conditions associated with employment are prevalent, and, if so, to undertake definitive measures to alleviate them.

The extent of medical surveillance for a given employee will vary greatly and be dependent upon:

- the nature of the research project in which they are involved
- the biological agents to which they are directly or potentially exposed
- certain additional factors relating to the current or previous health status of the individual.

Medical surveillance is provided without charge for any employee of Reed College whose job may result in potential exposure. For more information about this program contact the EHS.

3.1 Immunizations

In certain situations, personnel engaged in particular research activities would be immunized with appropriate vaccines, such as rabies, rubella and measles. Vaccines not commonly available will be obtained, whenever possible, for those engaged in specific research with potential exposure to the agent in question.

Vaccine	Recommendations
Rabies Vaccine	Recommended for all personnel entering laboratories or animal facilities with rabies vaccination entrance requirements.
Hepatitis B Vaccine	Recommended for persons working with human blood, body fluids or tissues.

Lyme Disease Vaccine	Recommended for persons working with the Lyme Disease agent or vectors in research laboratories, with animals, or in fieldwork.

In some cases, appropriate follow-up serum samples will be collected at periodic intervals to measure vaccine-induced antibodies when indicated.

3.2 Medical Restrictions

3.2.1 Pregnancy

It is recognized that exposure to certain infectious agents may adversely affect a fetus during pregnancy if the child-bearer is infected with the agent. Therefore, if pregnancy is possible while you are working in an infectious disease laboratory or laboratory engaged in work with infectious agents you should consult your Principal Investigator or supervisor.

Women that are pregnant or become pregnant are encouraged to inform their supervisors or Principal Investigators and their primary care physician. Employees are urged to discuss exposure issues with their supervisors or Principal Investigators regarding associated risks of research being conducted and pregnancy.

3.2.2 Reproductive Biological Hazards

The Environmental Health and Safety Office will offer confidential counseling to any individual of childbearing age working with reproductive pathogens or other potentially infectious materials. Reproductive biological hazards include, but are not limited to the following:

- Cytomegalovirus (CMV)
- Hepatitis B virus (HBV)
- Hepatitis E virus
- Human Immunodeficiency virus (HIV)
- Human parvovirus B19
- Rubella (German Measles)
- Lymphocytic Choriomeningitis virus
- Toxoplasma gondii (Toxoplasmosis)
- Listeria monocytogenes
- Varicella-zoster virus (chicken pox)

Whenever necessary, the Biosafety Officer must offer an opportunity to review work procedures in the lab to ensure that potential exposure is minimized. Consideration for reassignment to other tasks that don't involve exposure to the reproductive hazard (generally with actual pathogens, not necessarily for other potentially infectious materials such as blood or body fluids) should be given. Also, Investigators actively working with reproductive hazards must explain the risk assessment at time of hire.

3.2.3 Other Restrictions

Restrictions or recommendations will be made on an individual basis. Examples of conditions that might warrant special precautions are HIV infection, immunosuppressive conditions and drug therapies that suppress the immune system. Therefore, if you are suffering from any of the above conditions, you should inform your physician about the situation.

If you are immunocompromised, or have any other condition that might increase your risk factor working with biohazards and/or human etiological agents, you should consult with your physician and/or and the HCC for health recommendations, the Disability & Accessibility Resources Office, and work with your PI, lab instructor, supervising faculty member, and EHS, to develop additional safety protocols. In addition to these, it is recommended that your lab increase their Biosafety Level, as detailed by the BMBL, to respond to the increased risk. For more information, consult Appendix A.

4 Accidents

4.1 Emergency Procedures for Exposure Incidents

An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of an employee's duties. An employee who sustains a known or potential exposure incident must remove gloves and treat the affected area immediately by following the appropriate exposure incident response below.

4.1.1 Percutaneous Injury

Wash the affected area with antiseptic soap and warm water for 15 minutes.

4.1.2 Splash to Face

Flush affected area in eyewash for 15 minutes.

4.1.3 Aerosol Exposure

Hold your breath and immediately leave room. Remove Personal Protective Equipment (PPE) carefully. When removing PPE make sure to turn the exposed areas inward. Wash hands well with soap and water. Post spill sign on lab entry; lab should be evacuated for at least 30 minutes. PI must clear the lab for re-entry.

For extensive BL2 contamination (i.e. centrifuge incident) EHS must be notified and will assume responsibility, in conjunction with the PI, to clear the laboratory for re-entry.

4.2 Reporting Incident

The employee must report the incident to his/her supervisor. The supervisor must complete an Accident/Incident report (https://www.reed.edu/ehs/safety-information-and-programs/index/a/Accident-Incident-Report-Form_2015.pdf) documenting the route of exposure and the circumstances under which the incident occurred.

4.3 Medical Assistance

Reed College will provide the post-exposure evaluation and follow-up at no cost to employees who experience “exposure incidents”. The post-exposure monitoring periods are dependent on the type of exposure. This time period is related to the various incubation periods of the infectious agents.

4.4 Investigation of Laboratory Accidents

The Office of Environmental Health and Safety, in cooperation with the Principal Investigator and his or her staff, will conduct the necessary investigation of a laboratory accident. The goal of the investigation is the prevention of similar accidents as well as obtaining information concerning the circumstances and number of employees who have been exposed to the agent in question. In addition, the Office of Environmental Health and Safety, might institute further steps to monitor the health of those who may have been exposed to the agent in question

5 Risk Assessment and Risk Management

Responsibility for biosafety exists at all levels and is shared throughout the College. The Institutional Biosafety Committee (IBC) and Biosafety Officer (BSO) have the authority to administer the campus biosafety program. The Institutional Biosafety Committee establishes policies for the safe use of biohazards and for compliance with all applicable regulations. As an agent of the Committee, the Biosafety Officer disseminates pertinent information; consults with faculty, staff, students and visitors; and monitors for non-compliance.

The researchers who perform work with biohazards are perhaps the most important component of the biosafety program, as they must incorporate the biosafety requirements and safety precautions into all facets of their work.

The Principal Investigator is ultimately responsible for safety within the laboratory. An integral part of this responsibility is to conduct a review of proposed work to identify potential hazards (risk assessment) and to adopt appropriate safety procedures before initiation of the experiments (risk management).

Certain experiments require advanced registration and Institutional Biosafety Committee approval prior to initiation.

A risk assessment/risk management matrix has been prepared to illustrate key elements of the process (see below). Relevant sections providing additional details are indicated within the matrix. Information on the routes of exposure is included at the end of this section.

The five P's of risk assessment and risk management are:

- Pathogen – hazardous biological agent.
- Procedures – proposed experimental manipulations and safe work practices.
- Personnel – appropriate training and skills.
- Protective equipment – protective clothing and safety equipment.
- Place – laboratory design.

Consider the five P's in each facet of laboratory work. Properly conducted, risk assessment can help prevent exposure to biohazards and minimize the potential for laboratory-acquired infection. Remember that prior planning prevents poor performance.

5.1 Risk Assessment and Management Table

	Risk Assessment	Risk Management
Pathogen	Agent classification (See Appendix A) Routes of infection Infectious disease process Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors	Registration ▪ Biosafety Officer ▪ Biosafety Committee ▪ USDA – restricted agents ▪ CDC – select agents ▪ FDA/NIH – human gene therapy
Procedures	Aerosol risk: sonicating, centrifuging, homogenizing, blending, shaking, etc. Percutaneous risk: needles, syringes, glass Pasteur pipettes, scalpels, cryostat blade/knife, etc. Splash/splatter risk: pipetting, microbial loop, etc.	Written set of standard operating procedures (SOPs) with safety practices incorporated Adherence to basic biosafety principles Label labs, areas, and equipment housing BL2 or higher agents Conduct lab inspections to review practices and containment equipment Use trial experiments with non-infectious material to test new procedures/equipment

Personnel	Host immunity ▪ Neoplastic disease ▪ Infection ▪ Immunosuppressive therapy ▪ Age, race, sex, pregnancy ▪ Surgery (splenectomy, gastrectomy) ▪ Diabetes, Lupus Immunization Post-exposure prophylaxis Serum banking Attitude toward safety Open wounds, non-intact skin, eczema, dermatitis	Safety training Prior work experience with biohazards Demonstrated proficiency with techniques Prompt reporting of all exposure incidents, near misses, as well as signs and symptoms of related disease to PI and EHS Investigation/review of incidents/spills, etc. to prevent future occurrence
Protective Equipment	Protection (containment) for: ▪ Aerosols – respirable size particles (<5µm Droplets/splatter) ▪ Sharps	Personal protective equipment (PPE): ▪ Respirators – HEPA, N-99, N-95, etc. ▪ Face (eye, nose, mouth) protection – mask and safety glasses, or chin length face shield ▪ Solid front gown or lab coat ▪ Gloves Biosafety cabinets Centrifuge safety buckets/rotors
Place – Laboratory facility	Risk group/biosafety level requirements Aerosol risk Restricted access	Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open Labels Containment laboratory with directional airflow

5.2 Routes of Exposures

In order for biological agents to cause disease, they must first enter or invade the body in sufficient numbers. Routes of entry include oral, respiratory, parenteral, mucous membrane and animal contacts (bites, scratches). Once inside the body, biohazards must meet other requirements to cause disease; they must colonize and establish in body cells, tissues and/or organs, overcome the body's natural defense mechanisms and mutate or adapt to body changes.

Other factors contribute to an individual's susceptibility to the disease process. These include age, immunological state, occupation, physical and geographic environment and predisposing conditions (such as alcoholism and other drug abuse, pregnancy and diseases such as diabetes). It is difficult to determine a minimum infectious dose when discussing biohazards. The same dose of a pathogen may produce no disease symptoms in one individual but may cause serious or even fatal disease in another. There are microorganisms for which it is thought one organism entering the body is sufficient to invade and promote the disease process; the bacteria that causes tuberculosis is an example. For many pathogens, 10 to 100 or more organisms must enter the body to cause infection leading to disease. See the table below for additional information on routes of exposure.

5.2.1 Routes of Transmission for Infectious Agents in the Laboratory

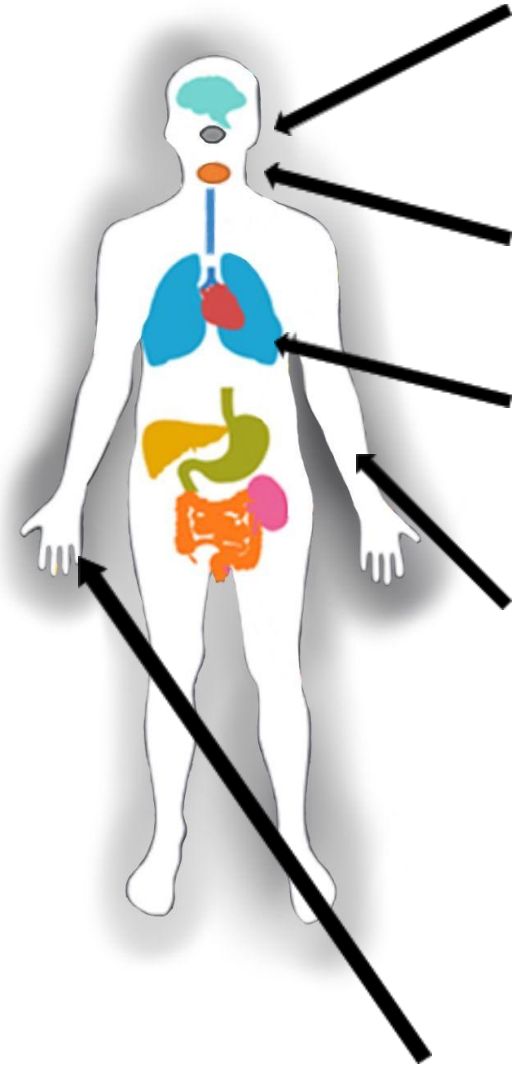
Route of Exposure	Protective Measures
Mucous Membranes. Exposure via the mucous membranes, eyes, nose, or mouth due to splash/splatter.	Achieve face protection by: ● wearing safety glasses and surgical mask or a full-face shield ● working in a biosafety cabinet or behind a protective shield ● following good microbiological practices
Inhalation. Breathing in respirable aerosols (particles <5µm) due to centrifuge leaks, spills, or aerosol-generating procedures such as pipetting, homogenizing, etc.	Avoid exposure to aerosols by: ● working in a biosafety cabinet ● using sealed rotors or canisters when centrifuging ● following good microbiological practices
Ingestion. Exposure from mouth pipetting or eating, drinking or smoking in the laboratory.	Prevent exposure via ingestion by: ● never eating, drinking or smoking in the laboratory ● always using mechanical pipettors ● following good microbiological practices

Percutaneous. Exposure through intact or non-intact skin via needle stick, puncture with a contaminated sharp object, animal scratch or bite, through wounds, abrasions, eczema	Prevent percutaneous injuries by: ● substituting plastic for glass ● using extreme caution with sharps ● discarding sharps immediately into a rigid leak-proof sharps container ● properly restraining animals ● wearing cut resistant gloves and sleeves ● covering non-intact skin with waterproof bandages and wearing double gloves
Contact (indirect exposure). Touching mucous membranes with hands that have been in contact with contaminated surfaces such as benches, phones, computers, etc. or hands that were not washed after working.	Prevent indirect exposure by: ● decontaminating work surfaces ● always washing hands when finished working or gloves have been compromised ● not touching face with gloves or non gloved hands (good personal hygiene) ● not applying cosmetics within the laboratory

Whenever in the laboratory always adhere to the basic biosafety principles:

- Do not eat, drink or smoke in the laboratory
- Always wash hands when finished working or gloves have been compromised
- Wear PPE within the laboratory. Be sure to remove PPE prior to leaving the laboratory
- Never mouth pipette, always use mechanical pipettors
- Use extreme caution when working with sharps
- Contain aerosols by using appropriate equipment
- Decontaminate work surfaces, spills and waste

Routes of Transmission for Infectious Agents



Mucous Membranes:
Exposures to mucous membranes
of the eyes, nose and mouth
through splashes or splatters.

Ingestion:
Mouth pipetting, eating, drinking, smoking in the lab.

Inhalation:
Breathing in respirable sized aerosols (<5µm), centrifuge leaks, spills, pipetting, etc.

Percutaneous:
Through intact or non-intact skin via
needlestick, puncture with contaminated
sharp object, animal scratch or bite,
through wounds, abrasions, or eczema.

Contact (indirect transmission):
Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment handles) or by failure to wash hands after working

5.3 Biosafety levels

The CDC and NIH have established biosafety levels for work with biohazardous materials in the publication Biosafety in Microbiological and Biomedical Laboratories (BMBL). The publication provides combinations of microbiological practices, laboratory facilities, and safety equipment as well as their recommended use in four biosafety levels (BL) of laboratory operation with selected agents infectious to humans. Also included in the BMBL is a parallel set of biosafety levels for research involving small laboratory animals.

Below is a summary of practices, equipment and facility requirements for agents assigned to biosafety levels 1–4 (BL 1–4). Additional information on biosafety levels may be found in Appendix B as well as in the BMBL, which is available at https://www.cdc.gov/labs/BMBL.html

Only work at biosafety levels 1-2 is currently permitted at Reed College.

5.3.1 Summary of Recommended Biosafety Levels for Infectious Agents

Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices.	None required.	Open bench top sink required.
2	Associated with human disease, hazards are auto inoculation, ingestion, mucous membrane exposure.	BSL-1 practice plus: ● Limited access; ● Biohazard warning signs; ● "Sharps" precautions; ● Biosafety manual defining any needed waste decontamination or medical surveillance policies.	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats; gloves; face protection as needed.	BSL-1 plus: Autoclave available.

3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.	BSL-2 practice plus: ● Controlled access; ● Decontamination of all waste; ● Decontamination of lab clothing before laundering; ● Baseline serum	Class I or II BCSs or other physical containment devices used for all manipulations of agents; PPE: protective lab clothing; gloves; respiratory protection as needed.	BSL-2 plus: ● Physical separation from access corridors; ● Self-closing, double door access; ● Exhausted air not recirculated; ● Negative airflow into laboratory.
4	Dangerous or exotic agents which pose high risk of life threatening disease, aerosol transmitted lab infections; or related agents with unknown risk of transmission.	BSL-3 practices plus: ● Clothing change before entering ● Showering on exit ● All material decontaminated on exit from facility.	All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air supplied, positive pressure personnel suit.	BSL-3 plus: ● Separate building or isolated zone; ● Dedicated supply/exhaust, vacuum, and decontamination systems; ● Other requirements outlined in BMBL.

Adapted from the Office of Health and Safety, Centers for Disease Control and Prevention.

6.0 Signs and Labels

6.1 Wall Signs

6.1.1 Biosafety Level

Entryways to research areas that handle BL2 materials, human blood or other potentially infectious materials must be posted with a BL2 biohazard sign that contains the universal biohazard symbol, the legend “Biohazard” and the term BL2.

6.2 Door Signs

BL2+ laboratories must have a biohazard door sign posted on all access doors.
The sign includes the international biohazard symbol, bears the legend "Biohazard", and identifies the name of the infectious agent, any special entrance requirements, and the name and phone numbers of the Principal Investigator or any other responsible persons. The following elements must be included on the door sign:

BIOHAZARD
(Name of infectious agent)
(Special entrance requirements)
(Name, telephone number of the Principal Investigator or other responsible person)

The door signs shall be fluorescent orange-red (or predominantly so) with lettering or symbols in a contrasting color.

6.3 Labels and Color-coding

Inside the facility, warning labels shall be affixed to containers of medical waste, refrigerators, freezers, incubators, and centrifuges containing BL2 agents, human blood or "other potentially infectious material". Other equipment such as waterbaths, sonicators, and biosafety cabinets do not require a permanent biohazard label if decontaminated after each use. In these situations, a biohazard label should be temporarily posted on the equipment while in use with human blood, other potentially infectious materials, or an infectious agent.

Warning labels shall also be affixed to other containers used to store, transport or ship BL2 agents, human blood or "other potentially infectious material". (Note: Shipping blood and “other potentially infectious material” not suspected of harboring an infectious agent may not require the biohazard warning label, just Exempt Human Specimen or Exempt Animal Specimen label, contact EHS or Mail Services). Labels required must have the international biohazard symbol and bear the legend "Biohazard" (see figure below).

BIOHAZARD

The labels shall be fluorescent orange-red (or predominantly so) with lettering or symbols in a contrasting color. Labels shall be affixed as close as feasible to the container by string, wire, adhesive, or any other method that prevents their loss or unintentional removal.

The use of warning labels may be waived if:

- waste is placed in red bags or red containers; or
- individual containers of blood or "other potentially infectious materials" are placed in a labeled secondary container during storage, transport, shipment or disposal.

6.4 Labeling Equipment Sent Out for Repair or Disposal

Contaminated and potentially contaminated equipment sent out for repair or disposal must be decontaminated as thoroughly as possible. Affix a tag to the equipment indicating when the equipment was decontaminated, what disinfectant was used, and the name of the person who performed the decontamination. Thorough decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. Decontaminate the equipment to the degree possible (flushing lines or wiping down the exterior) and affix a label to the equipment before sending it out for repair. The label must indicate what portions of the equipment remain contaminated and include the biohazard symbol as well as the legend "Biohazard". The label must convey this information to all affected workers (service representatives, manufacturer, etc.). Equipment tags can be obtained from the Office of Environmental Health and Safety.

7.0 Laboratory Practices

This section details information regarding hazards involved with certain laboratory practices and methods for preventing them. Prevention is an important element to biohazard control, and it is recommended that anyone working in a laboratory read this section carefully.

7.1 Human Factors and Attitudes in Relation to Laboratory Accidents

For the purpose of safety, an attitude can be defined as an accumulation of information and experience that predisposes an individual to certain behavior. Human factors and attitudes result in tendencies on the part of the individual to react in a positive or negative fashion to a situation, a person or an objective. Laboratory supervisors and Principal Investigators should understand the importance of attitudes and human factors in their own efforts to control biohazards in their laboratory. Some observations that may be of help to supervisors are listed below:

- The lack of accident perception ability is often a significant factor in laboratory accidents.
- Inflexibility of work habits, that tend to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.
- Working at an abnormal rate of speed is a significant causal factor.
- Intentional violations of regulations are a frequent cause of accidents. This is termed excessive risk taking.
- The performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
- Working when one is very tired is more likely to create a higher potential for accidents.
- Working at a well-organized and uncrowded laboratory bench will help in the prevention of lab accidents.

Each employee working with biohazardous agents must be consistently aware of the importance of the proper attitude in preventing accidents in the laboratory.

7.2 Biosafety Level 1

- Keep laboratory doors closed when experiments are in progress.
- Use procedures that minimize aerosols.
- Do not smoke, eat, drink or store food in BL1 areas.
- Wear laboratory gowns or coats when appropriate.
- Do not mouth pipette. Use mechanical pipetting devices.
- Avoid using hypodermic needles.
- Wash hands after completing experimental procedures and before leaving the laboratory.
- Disinfect work surfaces daily and immediately after a spill.
- Decontaminate all biological wastes before discarding them. Decontaminate other contaminated materials before washing, reuse, or discard.

- For off-site decontamination, package contaminated materials in closed, durable, leak-proof containers.
- Control insect and rodent infestations.
- Keep areas neat and clean.

7.3 Biosafety Level 2

- Keep laboratory doors closed.
- Post a universal biohazard label on equipment where infectious agents are used/stored.
- Allow only persons informed of the research to enter BL2 areas.
- Keep animals not used in BL2 experiment out of the laboratory.
- Do not smoke, eat, drink, store food or apply cosmetics in BL2 areas.
- Wear PPE (laboratory gowns or coats, gloves and full-face protection) when appropriate; do not wear PPE outside of the laboratory.
- Wash hands after removing PPE as well as before leaving laboratory.
- Change PPE when soiled or compromised.
- Do not mouth pipette. Use mechanical pipetting devices.
- Use procedures that minimize aerosol formation.
- Avoid using hypodermic needles.
- Substitute plastic for glass where feasible.
- Use biosafety cabinets to contain aerosol-producing equipment.
- Wash hands after completing experimental procedures and before leaving laboratory.
- Disinfect work surfaces daily and immediately after a spill.
- Report spills, accidents, near misses and disease symptoms related to laboratory acquired infection to the PI.
- Ensure that all biomedical waste containers are labeled with the biohazard symbol.
- Decontaminate all biological wastes before discarding them. Decontaminate other contaminated materials before washing, reuse, or discard.
- For off-site decontamination, package contaminated materials in closed, durable, leak-proof containers.
- Control insect and rodent infestations.
- Keep areas neat and clean.

7.4 Biosafety Level 2+

Biosafety level 2+ (BL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BL2 procedures. Generally, BL3 practices are mandated in a space designed for BL2 work. It is preferred that the BL2 laboratory be self contained with all equipment required for the experiment located within the laboratory. A biohazard door sign listing the agent in use, emergency contact, and entry requirements is posted on the door while BL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment has been decontaminated, the sign is removed and the laboratory is returned to standard BL2 or BL1 use.

All manipulations of BL2+ material are conducted in a class II biosafety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures. Additional requirements for work at BL2+ are listed in Appendix C. Please consult EHS and the IBC prior to initiating any work at BL2+.

7.5 Cell Culture

- Wear long sleeved gowns with knit cuffs and long gloves when working in the biosafety cabinet.
- Glassware and other contaminated items should be disinfected or autoclaved before washing, reuse or disposal.
- Glassware should be thoroughly cleaned and rinsed, by washing repeatedly with tap water and distilled water.
- Cell culture wastes must be decontaminated.
- Maintain a clean lab coat reserved solely for cell culture work.
- Avoid talking during culture manipulations as aerosols may be drawn into the work area.
- Place pipettes on a rack to avoid disrupting airflow when removed.
- Keep open tubes parallel to the airflow.
- After transferring inoculum, always recap vials.
- Do not place tubes on work surface.
- Discard empty tubes immediately.
- Work with one specimen at a time; recap before going to the next.
- Autoclave verification should be performed routinely.

7.6 Transport of Biohazards on Campus (between labs or buildings):

Must have two leak-proof containers, including the following:

- a sealed primary container
- a sealed secondary container
- absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
- a biohazard sticker on the outside of the secondary container with agentname
- lab address and phone number on the outside of the secondary container

Utilize plastic containers whenever feasible; avoid glass. Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags. If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.

Decontaminate the outside of the primary container before placing into the secondary container. Decontaminate the secondary container before leaving the laboratory.

7.7 Basic Microbiological Practices

7.7.1 Culture Plates, Tubes and Bottles

In the absence of definite accidents or obvious spillage, it is not certain that the opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means. Particular care is required when opening plates, tubes, or bottles containing fungi, for this operation may release a large number of spores. Such cultures should be manipulated in a biosafety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is re-suspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture of the colony. Following use of inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop or needle. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water of syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. Vented plastic Petri dishes, where the lid touches the rim at only three points, are less likely to offer this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent dispersal. If plates are obviously wet, they should be opened in the biosafety cabinet.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or

other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened. Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biosafety cabinet wearing gloves and a long sleeved laboratory garment.

Dried, infectious culture material may also collect at or near the rim or neck of culture tubes/flasks and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened in a biosafety cabinet.

7.7.2 Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created. Aerosol creation should be prevented or minimized; opening of ampoules should be done in biosafety cabinets. When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a biosafety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then wrap the ampoule in disinfected wetted cotton, and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw the contents into a fresh container. Some researchers may desire to use commercially available ampoules pre-scored for easy opening. However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, non-breakable leak-proof secondary containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel. The secondary container must be labeled with a biohazard label bearing the name of the infectious material.

7.7.3 Embryonated Eggs

Harvesting cultures from embryonated eggs is a hazardous procedure and leads to heavy surface contamination of the egg trays, shells, the environment, and the hands of the operator. It is essential that operations of this type be conducted in a biosafety cabinet. A suitable disinfectant should be at hand and used frequently.

7.8 Housekeeping

Well-defined housekeeping procedures and schedules are essential in reducing the risks associated with working with pathogenic agents and in protecting the integrity of the research program. This is particularly true in the laboratory operating under less than total containment concepts and in all areas used for the housing of animals, whether or not they have been intentionally infected. A well-conceived and well-executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminates in the event of inadvertent release of an etiologic agent. Less immediately evident are the benefits of establishing, among personnel of widely varying levels of education, some concepts of the nature and sources of contamination.

7.8.1 Objectives of Housekeeping

The objectives of housekeeping in the laboratory are to:

- Provide an orderly work area conducive to the accomplishment of the research program.
- Provide work areas devoid of physical hazards.
- Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems understudy.
- Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.
- Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that may:

- Harbor microorganisms potentially a threat to the integrity of the biological systems under investigation.
- Enhance the survival of microorganisms inadvertently released in experimental procedures.
- Retard penetration of decontaminates.
- Be transferable from one area to another on clothing and shoes.
- With sufficient buildup, become a biohazard as a consequence of secondary aerosolization by personnel and air movement
- Cause allergenic sensitization of personnel (e.g., to animal dander).

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other area in the laboratory has the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

7.8.2 Scope

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological materials are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding. The list below outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the laboratory complex must be viewed.

- Aisles
- Eyewashes
- Lab Entry and Exit Ways
- Bench Tops
- Floors
- Lab Equipment Cleanup
- Biosafety Cabinets
- Glassware
- Refrigerators
- Cold Rooms
- Hallways
- Supply Storage
- Deep Freezer Chests
- Incubators
- Waste Accumulations
- Dry Ice Chests
- Insect and Rodent Control
- Work Surfaces
- Instruments

7.8.3 Assignment of Responsibilities

Housekeeping in the laboratory is one avenue that leads to safely accomplishing the research program. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. The supervisor should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by a contracted cleanup crew; delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements; and the location of concentrated biological preparations, as well as contaminated equipment used in their preparation and application, will be known.

8.0 Personal Protective Equipment (PPE)

The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research operations and levels of risk associated with the research. While PPE is an important component of any Biosafety program, PPE is used with the understanding that PPE serves as a second line of defense. Good laboratory techniques, procedures and appropriate laboratory equipment are the primary barriers against potential exposure to hazardous agents.

8.1 Laboratory Clothing

A commonly used PPE item within the laboratory is special clothing. Both reusable and disposable clothing is available. Whichever is used, it must be durable, designed to provide protection and prevent exposure of the skin to harmful agents, as well as be compatible with the methods of decontamination employed.

Laboratory clothing serves to protect the wearer, the experiment, and environment against contamination. If proper precautions are not taken, contaminated clothing may carry infectious materials outside the laboratory and into other work areas, cafeterias, or the home. Infectious agents can remain viable on cotton and wool fabrics and be disseminated from these fabrics.

Some additional points:

- Overt exposure to agents at all levels of risk should be followed by immediate decontamination of the PPE and change into clean PPE to protect the worker, the experiments and the environment.
- Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.
- PPE worn within the laboratory should not be worn outside the laboratory.
- PPE should be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.
- All PPE should be decontaminated before being sent to the laundry or discarded. Treat contaminated areas of PPE with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
- Do not take PPE home to launder; select a laundry service that follows universal precautions.
- Change PPE as soon as feasible whenever it is compromised, soiled or torn.
- Wear appropriate sizes and keep an adequate supply of PPE available in the laboratory.
- Wash hands whenever PPE is removed.
- Do not touch door handles, elevator buttons, telephones, computers or other clean surfaces or items with gloved hands.
- Wear closed-toe shoes and long pants to guard against skin contamination or chemical exposure. Do not wear sandals or shorts in the laboratory.

8.1.1 Gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. Quality assurance is an important consideration.

No one glove can be expected to be satisfactory for all intended uses. Gloves may be fabricated of cloth, leather, natural and synthetic rubbers, or plastics. New formulations of synthetic rubber and plastic continue to be developed as research makes varied and changing demands on the protective capabilities of gloves. Changing applications lead to improved capabilities of impermeability, strength, flexibility, tactile sense and control.

Disposable (single use) gloves provide a barrier between infectious agents and the skin. Glove use is a basic precept of preventing infectious agent transmission. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common.

Gloves shall be removed and hands washed before exiting the laboratory. Use the one glove method, or an appropriate secondary container, when transporting materials through common use areas.

The Office of Environmental Health and Safety (EHS) can provide information on gloves needed for various tasks, such as working with animals, dry ice, heat, acids, etc. Consult EHS with details of your work to receive further information about the type and availability of gloves that will best meet your requirements.

Considerations for the selection and use of gloves:

- Gloves are not 100% leak-proof; change gloves periodically and when soiled and always wash hands after removing gloves or other PPE.
- Gloves will not prevent needle sticks or other puncture injuries.
- Check gloves for visible tears before use.
- Avoid wetting examination gloves as water or disinfectants will encourage wicking and leaking
- Do not reuse examination gloves; discard contaminated gloves in a biohazard bag immediately after use.
- Double glove or use household utility gloves when cleaning spills. Household utility gloves may be decontaminated and reused (replace when compromised.)

8.1.2 Procedure for Removing Gloves

Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

When removing PPE, remove lab coat or solid front gown first, then remove gloves (aseptically). Remove face protection last to avoid touching your face with contaminated hands. If wearing double gloves, remove outer gloves before removing lab coat or solid front gown.

8.1.3 Shoes

Shoes worn in the laboratory must be closed-toe. Protective shoes are required for certain work activities. When working with infectious agents it is advisable to wear shoe covers, which can be decontaminated (autoclaved) before disposal, over street shoes. For work in tissue culture laboratories it may be necessary to change from street shoes to specific laboratory shoes for protection of cultures from contamination.

8.1.4 Gowns, Lab Coats, Jumpsuits, Aprons and Other Protective Clothing

Gowns, lab coats and jumpsuits protect the wearer's clothing and skin from contamination. As with all PPE, the type of clothing needed depends on the task being performed and the degree of exposure anticipated.

Solid front wrap-around clothing offers better protection than pull-over type clothing or clothing with front closures. Lab coats are not 100% leak-proof; change PPE when soiled, and always wash your hands after removing any PPE. Lab coats or other protective clothing will not prevent needle sticks or other punctures. Spills and splashes occur most often in the chest or lap area. The contaminated surface must be touched during removal of a front closing jacket or lab coat. The contaminated portion often ends up in the wearer's face during removal of pullover clothing. Many workers prefer not to button up front closing jackets, which leaves street clothing exposed.

Long sleeved garments with snug fitting cuffs are preferred over open or short sleeves. Snug fitting cuffs prevent splashes, splatters and aerosols from making contact with exposed skin on the lower arms. Longer single-use gloves can be pulled over snug fitting cuffs to seal out any infectious materials.

Plastic, vinyl or rubber aprons are usually worn over other protective clothing when extra protection is desired. Aprons are necessary for protection against liquids spilling or splashing on clothing. It is recommended that appropriate aprons be worn to protect against the potential harmful effects of liquid waste.

8.1.5 Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid, to splash on the head, face and eyes or contact lenses. A variety of face shields, head covers/hoods, protective goggles, and lenses are available. The selection is dependent upon materials of construction, fit, comfort, and compatibility with the work and the overall facial area requiring protection.

Some of the considerations for selection and use of face and eye protection are indicated below:

- Face shields and hoods protect the face and the neck from flying particles and sprays of hazardous material; however, they do not provide basic eye protection against impacting objects.
- Shields should cover the entire face, permit tilting back to clean the face if desired, and be easily removed in the event of an accident.
- If an eye hazard exists in a particular operation or experiment, the soundest safety policy would be to require that eye or face protection, or both, be worn at all times by all persons entering or working in the laboratory.

8.2 Respiratory Protection

Protection of the respiratory system is a major concern of any Biosafety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to ten organisms, when inhaled, may cause infection. Particles with an effective aerodynamic diameter of between 0.5 and 5.0 μm (the respirable fraction) are most effective at penetration and retention in the deep pulmonary spaces. Particles larger than 5 micrometers are generally trapped in the upper respiratory tract and eventually cleared or swallowed.

Engineering controls, such as the use of Biosafety cabinets, should always be considered as a first line of defense against respiratory infection when working with infectious organisms. Respirators should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.

Respirators vary in design, application, and protective capability. Respirators can be placed into two categories:

- Air-purifying
- Supplied air

By far, the most commonly used respirators in laboratories are air-purifying respirators. These protect by purifying the existing breathing air through a filter (for particulates) or cartridge (for gasses and vapors). Dust masks that have been approved by NIOSH are also considered to be air purifying respirators. These are ranked by their filtering efficiencies and by whether they can be used in an environment containing oil aerosols. Approved dust masks will have one of the following designations – N95, N99, N100, R95, R99, R100, P95, P99, or P100. Proper selection of cartridges and respirators is very important and should not be made without input from the Office of Environmental Health and Safety. New regulations concerning respirators require initial and annual training and fit testing, and well as medical surveillance of all respirator wearers. Please make sure that the Office of Environmental Health and Safety is notified whenever the use of a respirator is being considered.

8.3 Selection of PPE

Use the following PPE to minimize exposure via mucous membrane OR non-intact skin:

For face protection, wear safety glasses and a mask, or a chin length face shield whenever splashing, splattering or droplets may be anticipated (any work with liquids on the open bench).

Gloves and a lab coat are worn to protect the skin and clothing from contact with potentially infectious materials. Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists. Consider double gloving when working with cultures of infectious agents or handling spills. Thicker household utility gloves can be worn for cleaning blood or BL2 spills. Utility gloves can be decontaminated and reused until the integrity of the glove is compromised. Temperature resistant gloves should be worn to protect hands from physical damage when working with very hot (autoclave) or cold (liquid nitrogen tank, -70°C freezer) materials.

Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin conditions or wounds. Avoid working with BL2 or other potentially infectious materials if non-intact skin cannot be adequately covered.

Solid front gowns provide more protection to clothing and skin than lab coats. Solid front gowns are worn for high hazard infectious agent work. The tight-fitting cuffs of the gown help to minimize wrist contamination.

Impervious lab coats, gowns or aprons are worn when heavy contamination or soiling is likely. Head covers are worn to protect the hair and scalp from splatter or droplets when working with heavy contamination or when contact with the head is likely. When choosing a head cover make sure it is impervious to liquids (some head covers are not impervious).

Shoe covers are worn over the shoes to protect shoes from contamination when working in heavily contaminated areas.

Gowns, head and shoe covers also help keep contaminants from entering the sterile area in clean rooms and surgical suites.

Use the following PPE to minimize exposure via cuts, slices, or scratches:

- Kevlar gloves and sleeves are cut resistant and will help guard against slices, scratches or cuts, but will not prevent direct puncture or needle stick injuries.
- Steel mesh gloves also protect against slices, cuts, and scratches but will not eliminate punctures.
- Neoprene and other abrasive resistant gloves are cut resistant, but significantly reduce dexterity.

Use the following PPE to minimize exposure via aerosols:

- HEPA filtered respirators (air purifying or powered air purifying) are worn to prevent exposure to potentially infectious aerosols when cleaning spills of concentrated infectious material or responding to centrifuge incidents. Employees who wear a respirator must enroll in the Reed's Respiratory Protection Program before using a respirator.

8.4 PPE Requirements Table

PPE	Biosafety Level 1	Biosafety Level 2
Gloves	Recommended to prevent skin or clothing contact with BL1 materials. Note: work that may involve radioactive materials or chemicals will require the use of a lab coat and gloves.	Required
Lab Coat	Recommended to prevent skin or clothing contact with BL1 materials. Note: work that may involve radioactive materials or chemicals will require the use of a lab coat and gloves.	Required
Face-Protection		Wear protective eyewear and surgical mask or chin length face shield whenever splashing, splattering or spraying is anticipated to prevent contact with mucous membranes of the eyes, nose and mouth. Researchers may choose to augment eye protection by performing experiments behind a protective splash shield.
Respiratory Protection	Not required.	Not required.

Other		Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons, and household rubber gloves will be recommended on a case by case basis. Generally, additional protective clothing is required whenever there is a high potential for splashing of potentially infectious material, such as organ harvesting or large spill response and clean up.

9.0 Laboratory Equipment

9.1 Biosafety Cabinets

Biosafety cabinets (BSCs), when used properly, provide a clean work environment for research. Biosafety cabinets offer personnel, product, and environmental protection. The BSC provides primary containment for infectious materials. The efficacy of BSCs depends upon the behavior of the operator and the orientation of the unit in the facility.

The BSC isolates biohazards from personnel by confining the biohazardous material in the unit. The BSC removes aerosolized biohazardous material by moving air through high efficiency particulate air (HEPA) filters. The intake air is filtered through a HEPA filter before entering the BSC work area. Exhaust air also passes through a HEPA filter. Aerosols generated in the work area of the BSC are contained within the BSC.

Operating Procedures for Class II Biosafety Cabinet:

- If used, turn off UV light; turn on fluorescent light and blower.
- Disinfect all interior surfaces with 70% ethanol or suitable disinfectant.
- Place items required for procedure into cabinet; do not obstruct grills.
- Wait 2-3 minutes for contaminants to purge from work area.
- Keep materials at least 4 inches inside work area.
- Work should proceed from clean to contaminated areas.
- After procedure, allow cabinet to run 2-3 minutes before removing materials.
- Wipe down all work surfaces with 70% ethanol or suitable disinfectant.
- Turn off fluorescent light and blower if desired.

Many BSCs are equipped with germicidal ultraviolet (UV) lamps. Time of exposure, distance, presence of dust or debris and UV lamp intensity can affect the germicidal effect of the UV lamp. The visible blue- violet glow of the UV lamp does not indicate there is germicidal effect. The UV lamp needs to be cleaned periodically to remove dust. UV lamps may damage eyes, skin, and laboratory equipment. UV lamps should be turned off while the room is occupied.
EHS discourages the use of UV lamps due to the potential damage resulting from UV lamp use.

9.2 Procedures for Centrifugation

All centrifugation shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. If a small centrifuge is used and centrifuge safety cups are not available, the centrifuge should be operated in the biosafety cabinet.

Each person operating a centrifuge should be trained on proper operating procedures.
Keep a logbook detailing operation records for centrifuges and rotors to assist in determining service requirements.

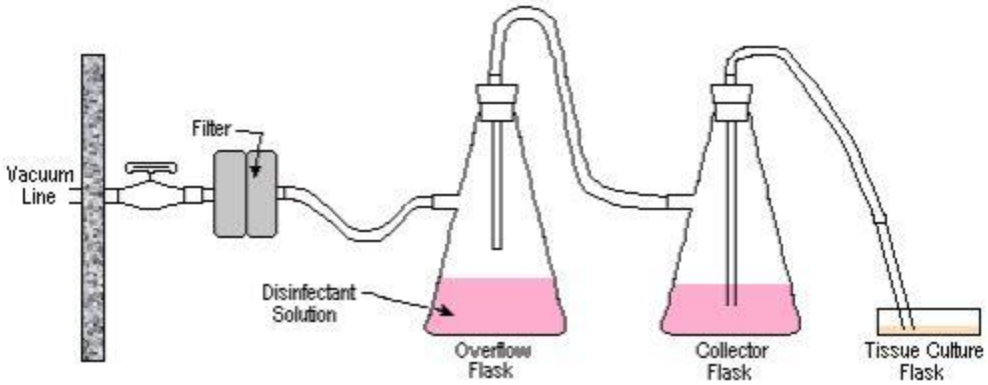
The following procedures for centrifugation are recommended:

- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the Biosafety cabinet. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- Always cap tubes before spinning.
- Place all tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Correct rough walls caused by erosion or adhering of matter and remove debris from the rubber cushions.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket.
- Never exceed safe rotor speed.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- Wait five minutes after the run before opening the centrifuge. This will allow aerosols to settle in the event of a breakdown in containment.
- Decontaminate safety carriers or rotors and centrifuge interior after each use.
- Open safety buckets or rotors in a biosafety cabinet. If the rotor does not fit in the biosafety cabinet, use the fume hood.

9.3 Vacuum Line Chemical Traps and Filters

Vacuum line chemical traps and filters prevent suction of infectious and non-infectious materials into the vacuum lines. Contact the Biosafety Officer for information regarding vacuum line filters. Considerations and Limitations of Vacuum Line Chemical Traps and Filters:

- Add full strength chemical disinfectant to chemical trap flasks. Allow the aspirated fluids to complete the dilution. For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard.
- Vacuum line filters shall be examined and replaced if clogged or if liquid makes contact with the filter. Used filters shall be discarded in the medical waste stream.



The above diagram depicts the proper set-up for vacuum lines that are used with potentially hazardous biological materials.

9.4 Syringes and Needles

The hypodermic needle is a dangerous instrument. To lessen the chance of accidental injection, aerosol generation, or spills, the use of syringes should be avoided when alternate methods are available. For example, use a blunt needle or cannula on the syringe for oral or intranasal inoculations and never use a syringe and needle as a substitute for a pipette in making dilutions.

The following practices are recommended for hypodermic needles and syringes when used for parenteral injections:

- Use the syringe and needle in a Biosafety cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.
- Examine glass syringes for chips and cracks, and needles for barbs and plugs. This should be done prior to sterilization before use. Use needle-locking syringes only, and be sure that the needle is locked securely into the barrel. Replace glass syringes with plastic disposable syringes whenever possible.
- Whenever possible use safer needle systems.
- Wear latex gloves for all manipulations with needles and syringes.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with an appropriate disinfectant, or into a small bottle of sterile cotton.
- Do not use the syringe to forcefully expel a stream of infectious fluid into an open vial for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the syringe is held below the surface of the fluid in the tube.
- If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in the transfer of infectious material to the fingers.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pledget may be used and immediately discarded into a biohazard bag.
- When inoculating animals, position the hand that is holding the animal “behind” the needle or use a pair of forceps to hold the animal in order to avoid puncture wounds.
- Be sure the animal is properly restrained prior to the inoculation and be on the alert for any unexpected movements of the animal.
- Before and after injection of an animal, swab the injection site with an appropriate antiseptic.
- Discard syringes into a needle box. DO NOT bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable, use a one-handed method. Discard all syringes in an appropriate biohazardous sharps container.

9.5 Pipettes

Never suction or pipette by mouth; always use some type of pipetting aid when pipetting infectious materials. Preferably, all activities should be confined to a biosafety cabinet. Pipetting of toxic chemicals should be performed in a chemical fume hood.

Infectious or toxic materials should never be forcefully expelled from a pipette. Mark-to-mark pipettes are preferable to other types because they do not require expulsion of the last drop. Infectious or toxic fluids should never be mixed by bubbling air from a pipette through the fluid. Infectious or toxic fluids should never be mixed by alternate suction and expulsion through a pipette. Discharge from a pipette should be as close as possible to the fluid or agar level, and the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.

Pipettes used for transferring infectious or toxic materials should always be plugged with cotton, even when safety pipetting aids are used.

Avoid accidentally dropping infectious or toxic material from the pipette onto the work surface. Place a disinfectant dampened towel or other absorbent material on the work surface, and autoclave before discard or reuse. Plastic backed bench paper is suitable for this purpose. Contaminated pipettes should be placed horizontally into a pan or tray containing enough suitable disinfectant, such as hypochlorite, to allow complete immersion of the pipettes. Pipettes should not be placed vertically in a cylinder that, because of its height, must be placed on the floor outside the biosafety cabinet. Removing contaminated pipettes from the biosafety cabinet and placing them vertically in a cylinder provides opportunity for dripping from the pipette onto the floor, or the rim of the cylinder, thereby creating an aerosol, and the top of the pipettes often protrude above the level of disinfectant.

Place discard pans for used pipettes within the biosafety cabinet.

After suitable contact time, excess disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together, and replaced by a clean pan with fresh disinfectant.

9.6 Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding, or disrupting biohazardous materials. Even the use of a mortar and pestle can be a hazardous operation. Other devices that may produce aerosols are ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers.

Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Wherever sonicators are used in the cleaning process; such as in dishwashers, animal cage washers, etc.; all items should be sterilized prior to cleaning.

The laboratory practices generally required when using equipment that may generate aerosols with biohazardous materials are as follows:

- Operate blending, cell disruption, and grinding equipment in a Biosafety cabinet;
- Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leak-proof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline, or methylene blue solution is recommended prior to use;
- If the blender is used with infectious material, place a towel moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use;
- Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break;
- Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product;
- Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud;
- Grinding of infected tissues or materials with any open device is best done within a Biosafety cabinet.

9.7 Lyophilizing

Specimens shell-frozen in ampoules are dried on a vacuum manifold or in a chamber-type drier at low negative pressure. If the glass neck of the ampoule is sealed off while the ampoule is still under vacuum, it may cause implosion, either during the sealing or later when the evacuated ampoule is being opened. To avoid this, after drying is completed, and before sealing is done, bring the pressure within the ampoule back to normal by gradually introducing dry nitrogen, avoiding turbulent disturbance of the dry product.

The narrow or constricted neck of the ampoule is contaminated if the specimen is allowed to run down the wall of the neck during filling. Subsequently, when the ampoule is sealed with a torch, the dried material on the wall becomes charred or partially decomposed; residues of this material may adversely affect the dried material when it is reconstituted. To avoid this, a syringe with a long cannula or a Pasteur-type pipette should be used to fill the vial. Do not allow the delivery end of the cannula or pipette to touch the neck of the vial.

All ampoules used for freeze-drying of cultures, toxins, or other biohazardous material should be fabricated of Pyrex-type glass. This type of glass requires a high-temperature torch using an air-gas or oxygen-gas mixture for sealing. These hard glass ampoules are much less apt to form gas bubbles that burst inwardly during sealing under vacuum than the soft glass ampoules and are more resistant to breakage during handling and storage.

The filling of ampoules and vials with infectious specimens, the subsequent freeze-drying, and sealing or closing of ampoules and vials in the preparation of dry infectious specimens should be performed in a biosafety cabinet. The same is true for the preparation of ampoules and vials containing liquid specimens not subject to freeze-drying.

Safety precautions to be taken will depend on the agents, equipment, and containment available. Therefore, before initiating this procedure, the Principal Investigator should work out the protocol for each machine in consultation with the Biosafety Officer. All persons using the procedure must then follow the protocol.

9.8 Miscellaneous Equipment (Waterbaths, Cold Storage, Shakers)

Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold-water baths, 70% propylene glycol is recommended. Sodium azide should not be used as a bacteriostatic. It creates a serious explosion hazard.

Deep freeze, liquid nitrogen, and dry ice chests as well as refrigerators should be checked, cleaned out periodically to remove any broken ampoules, tubes, etc. containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deep freezers should be properly labeled. Security measures should be commensurate with the hazards.

The degree of hazard represented by contaminated liquid nitrogen reservoirs will be largely dependent upon the infectious potential of the stored microorganisms, their stability in liquid nitrogen, and their ability to survive in the airborne state. Investigations suggest that storing tissue culture cell lines in containers other than sealed glass ampoules might result in potential inter-contamination among cell lines stored in a common liquid nitrogen repository.

Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because of the fragility of the membrane and other factors, such filtrates cannot be handled as non- infectious until culture or other tests have proved their sterility.

Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw-capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.

No person should work alone on an extremely hazardous operation.

10 Decontamination and Disposal Procedures

10.1 Decontamination Methods

Physical and chemical means of decontamination fall into three main categories: heat, liquid decontaminants, and vapors and gasses.

10.1.1 Heat

The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121°C under pressure in the autoclave is the most convenient method of rapidly achieving sterility under ordinary circumstances. Dry heat at 160°C to 170°C for periods of two to four hours is suitable for destruction of viable agents on an impermeable non-organic material such as glass, but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat for decontamination. Incineration serves as an efficient means of disposal for human and animal pathological wastes.

The hazards of handling hot solids and liquids are reasonably familiar. Laboratory personnel should be cautioned that steam under pressure could be a source of scalding jets if the equipment is misused. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too soon after treatment. This may cause a sudden and violent boiling of contents from the containers that can splash scalding liquids onto personnel handling the containers. See the autoclave safety poster in the Poster Section of this manual.

10.1.2 Liquid Decontaminants

In general, the liquid decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems.

There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, contact time, pH, the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in the effectiveness of decontamination. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids and alkalis, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines. Unfortunately, the

more active the decontaminant, the more likely it will possess undesirable characteristics such as corrosivity. None is equally useful or effective under all conditions for all infectious agents.

Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned to the task of making up use-concentrations from stock solutions must be informed of the potential hazards and trained in the safe procedures to follow and appropriate personal protective equipment to use as well as the toxicity associated with ocular, skin and respiratory exposure.

10.1.3 Vapors and Gasses

A variety of vapors and gasses possess decontamination properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in a closed system and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating Biosafety cabinets and associated air- handling systems and air filters; bulky or stationary equipment that resists penetration by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; and rooms, buildings and associated air-handling systems.

Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas. In preparing use-dilutions and when applying them, personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary. Mutagenic potential has been attributed to ethylene oxide; toxic and hypersensitivity effects are well documented for formaldehyde.

Use of formaldehyde and ethylene oxide is monitored closely by the EHS.

10.2 Autoclave Procedure

Moist heat causes the denaturation of proteins at lower temperatures and shorter times than dry heat. One of the most effective physical decontamination controls is steam sterilization (autoclave), which generates moisture, and high temperature pressurized steam within a sealed chamber. Autoclaves can sterilize all items that are heat stable. In gravity autoclaves, a cycle of 250°F (121°C) at 15 to 18 pounds per square inch (psi) of pressure for one hour may be required for decontamination. In the newer vacuum autoclaves, decontamination may require a cycle of 270°F (132°C) at 27 to 30 psi for 45 minutes.

A biological indicator should be used to verify proper autoclave operation.

Personal protection equipment (PPE) such as rubberized aprons, full-face shields and heat and liquid resistant gloves must be worn when operating autoclaves.

Position items in the autoclave to allow steam penetration into all items to be decontaminated. Materials in tightly sealed or stoppered containers may not be effectively decontaminated and may become dangerously pressurized causing injury when removed from the autoclave. Items containing chemicals such as phenol or chloroform should not be placed in an autoclave. See the autoclave safety poster in the Poster Section of this manual.

10.3 Characteristics of Chemical Decontaminants

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, or liquid concentrates. These may be added to water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperature are useful as space-penetrating decontaminants. Others become gases at elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways:

- Coagulation and denaturation of protein, or
- Lysis, or
- Binding to enzymes or inactivation of an essential enzyme by oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants may be altered substantially by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending on how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay system employed. Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms rather than failure of the decontaminant to act. If an item is placed in a liquid decontaminant, tiny bubbles are visible on the surface of the item. The area under the bubbles is dry and microorganisms in these dry areas will not be affected by the decontaminant. If there are spots of grease, rust or dirt on the item, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful. A decontaminant should have, and most do have, incorporated surface-active agents.

10.3.1 Properties of Some Common Decontaminants

Alcohol

Ethyl or isopropyl alcohol in a concentration of 70-85% by weight is often used; however, both lose effectiveness at concentrations below 50% and above 90%. Alcohols denature proteins and are somewhat slow in germicidal action. However, alcohols are effective decontaminants against lipid-containing viruses. A contact time of ten minutes is generally employed in efficacy

tests with disinfectants. Due to the high evaporation rate of alcohols, repeated applications may be required to achieve the required ten-minute contact time for decontamination. Because of this, the OSHA Bloodborne Pathogens Standard does not recognize alcohol as an effective decontaminant for surfaces.

Isopropyl alcohol is generally more effective against vegetative bacteria; ethyl alcohol is a more virucidal agent.

Formaldehyde

Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration referred to as formalin, or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures, and the pungent, irritating odors make formaldehyde solutions difficult to use in the laboratory. Formaldehyde vapor generated from solution is an effective space decontaminant for buildings or rooms, but in the vapor state in the presence of water tends to polymerize on surfaces to form paraformaldehyde, which is persistent and unpleasant. Heating paraformaldehyde to depolymerize it can liberate formaldehyde gas. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than is the case in the vaporstate.

Phenols

Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. Phenolic compounds are effective decontaminants against some viruses, fungi, and vegetative bacteria, including rickettsiae. Phenolics are not effective in ordinary use against bacterial spores.

Quaternary Ammonium Compounds or Quats

After 40 years of testing and use, there is still considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid- containing viruses. The Quats will attach to protein so that dilute solutions will quickly lose effectiveness in the presence of proteins. Quats tend to clump microorganisms and are neutralized by anionic detergents such as soap. They have the advantages of being nontoxic, odorless, stable, non- staining, non-corrosive to metals, and inexpensive.

Chlorine

This halogen is a universal decontaminant active against many microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in the presence of protein. Free available chlorine is the active element. It is a strong oxidizing agent and corrosive to metals. Chlorine solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25%, or 52,500 ppm, available chlorine. If diluted 1 to 100, the resulting solution will contain 525 ppm of available chlorine, and, if a nonionic detergent is added in a concentration of about 0.7%, a very good decontaminant is created.

Iodine

The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants for laboratory use are the iodophors, with Wescodyne being perhaps the most widely used. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water (25 ppm available iodine) to 3 oz. in 5 gal. of water (75 ppm available iodine). The small amount of free iodine available in this range can rapidly be taken up by extraneous protein that may be present. Clean surfaces or clear water can be effectively treated with 75-ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For iodophors such as Wescodyne, it is critical that the manufacturer's written instructions are followed. Higher concentrations of iodophors are actually less effective, as the iodine is bound to itself or the carrier molecule. For washing the hands or for use as a sporicide, it is recommended that Wescodyne be diluted 1 to 10 in 50% ethyl alcohol (a reasonably good decontaminant itself.) This will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

10.3.2 Selecting Chemical Disinfectants

No single chemical disinfectant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical disinfectants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

- What is the target organism(s)?
- What disinfectants, in what form, are known to, or can be expected to, inactivate the target organism(s)?
- What degree of inactivation is required?
- In what menstruum is the organism suspended (i.e. simple or complex, on solid or porous surface, and/or airborne)?

- What is the highest concentration of organisms anticipated to be encountered? • Can the disinfectant, either as a liquid, vapor, or gas, be expected to contact the organism and can effective duration of contact be maintained?
- What restrictions apply with respect to compatibility of materials?
- What is the stability of the disinfectant in use concentrations, and does the anticipated use situation require immediate availability of the disinfectant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the laboratory is the organism(s) under investigation. Laboratory preparations or cultures usually have titers in excess of those normally observed in nature. Inactivation of these materials presents other problems since agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active moieties of chemical disinfectants. Such interference with the desired action of disinfectants may require higher concentrations and longer contact times than those shown to be effective in the test tube. Similarly, a major portion of the contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information with which to predict the probable virulence of these more resistant cells. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting disinfectants and procedures for their use.

Organisms exhibit a range of resistance to chemical disinfectants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid-containing viruses are relatively susceptible to chemical disinfection. The non-lipid-containing viruses and bacteria with a waxy coating, such as tubercle bacillus, occupy a mid-range of resistance. Spore forms and unconventional (slow) viruses are the most resistant.

A disinfectant selected on the basis of its effectiveness against organisms on any range of the resistance scale will be effective against organisms lower on the scale. Therefore, if disinfectants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other organism generated by laboratory operations, even in higher concentrations, would also be inactivated.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in the table on the following pages. Practical concentrations and contact times that may differ markedly

from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstruums. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. Individual investigators should conclusively determine the efficacy of any of the disinfectants. It is readily evident that each of the disinfectants has a range of advantages and disadvantages as well as a range of potential for

inactivation of a diverse micro flora. Equally evident is the need for compromise as an alternative to maintaining a veritable “drug store” of disinfectants.

10.3.3 Characteristics of Some Liquid Disinfectants Table

Liquid Disinfectant Category	Use Dilution	Inactivates				Important Characteristics											Applications				
		Vegetative Bacteria	Lipoviruses	Non-Lipid Viruses	Bacterial Spores	Effective Shelf Life > 1 week[c]	Corrosive	Flammable	Explosion Potential	Residue	Inactivated by Organic Matter	Compatible with Optics[d]	Skin Irritant	Eye Irritant	Respiratory Irritant	Toxic[e]	Work Surfaces	Dirty Glassware	Portable Equip. Surface decon.	Fixed equip. Surface decon.	Liquid discard
Quat. Ammon.	0.1–2.0%	+	+			+					+	+	+	+		+	+	+	+	+	
Phenolic Cpds	1.0–5.0%	+	+	b		+	+			+			+	+		+	+	+	+	+	
Chlorine Cpds	500 ppm[a]	+	+	+	+		+			+	+		+	+	+	+	+	+	+	+	+
Iodophor	25–1600 ppm[a]	+	+	+	+	+	+			+	+		+	+		+	+	+	+	+	
Alcohol, Ethyl	70-85%	+	+	b		+		+						+		+	+	+	+	+	
Alcohol, Isopropyl	70-85%	+	+	b		+		+						+		+	+	+	+	+	
Formaldehyde	0.2–8.0%	+	+	+	+	+				+			+	+		+	+	+	+	+	
Glutaraldehyde	2%	+	+	+	+	+				+		+	+	+		+	+	+	+	+	

a. Available halogen
b. Variable results dependent on virus
c. Protected from light and air
d. Usually compatible, but consider interferences from residues and effects on associated materials such as mounting adhesives
e. By skin or mouth or both — refer to manufacturer’s license

Please note that a contact time of ten minutes is generally used in efficacy testing of disinfectants

11.0 Spill Response

This section outlines the basic procedures for dealing with some of the biological spills that you may encounter in your research laboratory. All lab personnel should refer to the relevant spill response procedures before initiating their experiments.

11.1 Composition of a Basic Spill Kit

Microbiological and biomedical research laboratories should prepare and maintain a biological spill kit. A spill kit is an essential safety item for labs working with microbiological agents classified at Biosafety Level 2 or higher and for groups working with large volumes (> 1 liter) of Biosafety Level 1 material. A basic spill kit should include:

- Concentrated household bleach
- A spray bottle for making 10% bleach solutions
- Forceps, autoclavable broom and dust pan, or other mechanical device for handling sharps
- Paper towels or other suitable absorbent
- Biohazard autoclave bags for the collection of contaminated spill cleanup items
- Utility gloves and medical examination gloves
- Face protection (eyewear and mask, or full face shield)

Representatives from the EHS are available if you have any questions regarding biological spill response procedures or decontamination.

11.2 Biosafety Level 1 (BL1) Spill

Notify others in the area, to prevent contamination of additional personnel and environment. Remove any contaminated clothing and wash exposed skin with disinfectant.

11.2.1 Clean-up of BL1 Spill

- Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill allowing it to mix with spilled material.
- Allow suitable contact time.
- Pick up any pieces of broken glass with forceps and place in a sharps container.
- Discard all disposable materials used to clean up the spill into a biohazard autoclave bag. Wash hands with soap and hand-washing disinfectant

11.3 Biosafety Level 2 (BL2) Spill

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with a warning sign.
- Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag. Wash all exposed skin with soap and water.
- Inform Supervisor, and, if assistance is needed, consult EHS.

11.3.1 Reporting of Spills Involving rDNA Materials

Spills or accidents in BSL2 laboratories involving recombinant DNA materials resulting in an overt exposure must be immediately reported to EHS.

EHS will assist in reporting the incident to the NIH Office of Biotechnology Activities (OBA), as required under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf).

11.3.2 Clean-up of BL2 Spill

- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory.
- Assemble cleanup materials (disinfectant, paper towels, biohazard bags, and forceps).
- Put on protective clothing (lab coat, face protection, utility gloves, and booties if necessary).
- Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask.
- Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least a 20-minute contact time.
- Pick up any sharp objects with forceps and discard in a sharps container. Soak up the disinfectant and spill using mechanical means, such as an autoclavable broom and dustpan, since there may be sharps under the paper towels, and place the materials into a sharps container. Smaller pieces of glass may be collected with cotton or paper towels held with forceps. If no sharps were involved in the spill, discard the materials into an autoclave bag.
- Wipe surrounding areas (where the spill may have splashed) with disinfectant.
- Soak up the disinfectant and spill, and place the materials into a biohazard bag.
- Spray the area with 10% household bleach solution and allow to air-dry (or wipe down with disinfectant-soaked towels after a 10-minute contact time). Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.

- Wash hands and exposed skin areas with disinfectant or antiseptic soap and water.

11.3.3 Blood Spills

For blood or other material with a high organic content and low concentration of infectious microorganisms:

- Wear gloves, eye protection, and a lab coat.
- Absorb blood with paper towels and place in a biohazard bag. Collect any sharp objects with forceps or other mechanical device and place in a sharps container.
- Using a detergent solution, clean the spill site of all visible blood.
- Spray the spill site with 10% household bleach and allow to air-dry for 15 minutes.
- After the 15-minute contact time, wipe the area down with disinfectant-soaked paper towels.
- Discard all disposable materials used to decontaminate the spill and any contaminated personal protective equipment into a biohazard bag.
- Wash your hands.

11.4 Spill of a Biohazardous Radioactive Material

A biohazardous spill involving radioactive material requires emergency procedures that are different from the procedures used for either material alone. Use procedures that protect you from the radiochemical while you disinfect the biological material.

Before any clean up, consider the type of radionuclide, characteristics of the microorganism, and the volume of the spill. Contact the EHS for isotope cleanup procedures.

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post a warning sign.
- Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag labeled with a radioactive materials label or a radioactive waste container labeled with a biohazard label.
- Wash all exposed skin with disinfectant, following it with a three-minute water rinse.
- Inform supervisor and EHS of spill, and monitor all exposed personnel for radiation. If assistance is needed in handling the microorganism, contact EHS.

11.4.1 Clean-Up of a Biohazardous Radioactive Material

Do Not use bleach solutions on iodinated material, radioactive gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.

Do Not autoclave the waste unless EHS approves this action. If waste cannot be autoclaved, add additional disinfectant to ensure biological decontamination of all the materials.

- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble cleanup materials (disinfectant, autoclavable containers, forceps, towel, and sponges), and confirm with EHS that it is safe to enter the lab.
- Put on protective clothing (gown, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA-filtered respirator instead of a surgical mask.
- Cover the area with disinfectant-soaked towels, and carefully pour disinfectant around the spill.
- Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least 20 minutes contact time.
- Handle any sharp objects with forceps. Wipe surrounding areas, where the spill may have splashed, with disinfectant.
- Soak up the disinfectant and spill, and place the biologically decontaminated waste, along with all contaminated protective clothing, into an approved radiation container. Contaminated protective clothing must also be biologically decontaminated prior to disposal as radioactive waste.
- Wash hands and exposed skin areas with disinfectant; monitor personnel and spill area for residual radioactive contamination.

If skin contamination is found, repeat decontamination procedures.

If the spill area has residual activity, determine if it is fixed or removable and handle accordingly.

11.4.2 Discarding items contaminated with radioactive materials:

- Place the contaminated item(s) on absorbent paper.
- Spray disinfectant (10% household bleach) on the contaminated areas and allow 20 minutes contact time.
- Wrap the item(s) inside the paper and dispose of as radioactive waste

12.0 Select Agents

Select agents are materials that have been identified by the U.S. Government as agents with potential for use in biological terrorism or warfare. The U.S. Centers for Disease Control and Prevention (CDC), and the Animal Plant Health Inspection Service (APHIS), through the United States Department of Agriculture (USDA), regulate select agents in the United States and its territories. Select agents include human, animal, and plant pathogens, high-risk toxins of biological origin, and prions.

The current list of select agents is below, and can also be accessed online at https://www.selectagents.gov/

The federal select agent regulations were updated and changes have taken effect as of October 5, 2022. The most significant changes are a policy statement for Large Animal Study-Related Activities with B. abortus and B. suis Using Outdoor Containment Spaces, and an update of the select agents and toxins list to include SARS-CoV/SARS-CoV-2 chimeric viruses.

12.1 Possession, Use, or Transfer of Select Agents

In order to possess, use, send or receive Select Agents, an institution and each individual who will have access to the Select Agent(s) must first satisfy the following requirements. Each requirement must be approved prior to possession, use or transfer.

- Register with the applicable U.S. Governing bodies (CDC, APHIS, and/or USDA) through the Reed Office of Environmental Health & Safety (EHS).
- Official authorization granted for each individual requesting access to Select Agents provided by the U.S. Federal Bureau of Investigation, the applicable U.S. Governing body, and Reed College.

Please note that violations of Select Agent rules and regulations can lead to severe criminal or civil penalties. Imprisonment and fines up to $250,000.00 may be levied against individuals who are found in violation of these laws.

12.2 List of Select Agents and Regulated Toxins

The current list of Select Agents below is current as of April 28th, 2023.
The Select Agent List is available online at: https://www.selectagents.gov/sat/list.htm
Select Agent Regulations may be found at: https://www.selectagents.gov/regulations/index.htm
The list of exclusions can be found at: https://www.selectagents.gov/sat/exclusions/index.htm

HHS Select Agents and Toxins

- Abrin
- *Bacillus cereus* Biovar *anthracis*
- Botulinum neurotoxins*
- Botulinum neurotoxin producing species of *Clostridium**
- Conotoxins (Short, paralytic alpha Conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
- *Coxiella burnetii*
- Crimean-Congo haemorrhagic fever virus
- Diacetoxyscirpenol
- Eastern Equine Encephalitis virus
- Ebola virus*
- *Francisella tularensis**
- Lassa fever virus
- Lujo virus
- Marburg virus*
- Mpox virus
- Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
- Ricin
- *Rickettsia prowazekii*
- SARS-associated coronavirus (SARS-CoV)
- SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors
- Saxitoxin
- **South American Haemorrhagic Fever viruses:**
    - Chapare
    - Guanarito
    - Junin
    - Machupo
    - Sabia
- Staphylococcal enterotoxins T-2 toxin
- Tetrodotoxin
- **Tick-borne encephalitis complex (flavi) viruses**
    - Far Eastern Tick-borne encephalitis
    - Siberian subtype
- Kyasanur Forest disease virus
- Omsk Hemorrhagic Fever virus
- Variola major virus (Smallpox virus)*
- Variola minor virus (Alastrim)*
- Yersinia pestis*

Overlap Select Agents and Toxins

- Bacillus anthracis*
- Bacillus anthracis Pasteur strain
- Brucella abortus
- Brucella melitensis
- Brucella suis
- Burkholderia mallei*
- Burkholderia pseudomallei*
- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Venezuelan Equine Encephalitis virus

USDA Veterinary Services (VS) Select Agents and Toxins

- African horse sickness virus
- African swine fever virus
- Avian influenza virus (highly pathogenic)
- Classical swine fever virus
- Foot-and-mouth disease virus*
- Goat pox virus
- Lumpy skin disease virus
- *Mycoplasma capricolum*
- *Mycoplasma mycoides*
- Newcastle disease virus (virulent)
- Peste des petits ruminants virus
- Rinderpest virus*
- Sheep pox virus
- Swine vesicular disease virus

USDA Plant Protection and Quarantine (PPQ) Select Agents and Toxins

• Peronosclerospora philippinensis (Peronosclerospora sacchari)
• Phoma glycinicola (formerly Pyrenochaeta glycines)
• Ralstonia solanacearum
• Rathayibacter toxicus
• Rathayibacter toxicus
• Sclerophthora rayssiae
• Synchytrium endobioticum
• Xanthomonas oryzae
* Denotes Tier 1 Agent

12.3 Tier 1 Select Agents

A subset of select agents and toxins have been designated as Tier 1 because these biological agents and toxins present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety:

Tier 1 Select Agents and Toxins		
HHS Agents and Toxins	**Overlap Agents**	**USDA Agents**
• Botulinum neurotoxins • Botulinum neurotoxin-producing species of *Clostridium* • Ebola virus • *Francisella tularensis* • Marburg virus • Variola major virus (Smallpox virus) • Variola minor virus (Alastrim) • *Yersinia pestis*	• *Bacillus anthracis* • *Bacillus cereus var. anthracis* • *Burkholderia mallei* • *Burkholderia pseudomallei*	• Foot-And-Mouth Disease virus • Rinderpest virus

Entities that possess, use, or transfer Tier 1 select agents and toxins must adhere to additional personnel screening and ongoing personal screening requirements. Additional physical requirements are also required for Tier 1 Select Agents.

12.4 Permissible Toxin Amounts

The following toxins are not regulated if the amount under the control of a Principal Investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the following table.

HHS Toxins [§73.3(d)(3)]	***Amount***
Abrin	1,000 mg
Botulinum neurotoxins (BoNT)	1.0 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg
Saxitoxin (STX)	500 mg
Staphylococcal Enterotoxins (SE) (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin (TTX)	500 mg

Source: https://www.selectagents.gov/compliance/guidance/toxin/list.htm

12.4.1 Toxin due Diligence Requirements

All Reed Principal Investigators in possession of ANY QUANTITY of the 9 Select Agent Toxins listed above must retain a record of ALL transfers of ANY QUANTITY of these toxins outside their laboratory. The required documentation must include the following information:

- Name of the recipient
- Toxin and quantity transferred
- Purpose of use (knowledge of recipient's legitimate need for the toxins)

12.4.2 Reporting Suspected Violations or Suspicious Activity

If a Reed Principal Investigator in possession of ANY QUANTITY of the 9 Select Agent Toxins listed above detects suspicious activity associated with a request for toxin or suspicious activity associated with a shipped toxin, s/he/they must immediately notify Reed College and the Federal Select Agent program at the contact information below:

OIG Hotline contact information:

Voice: 1-800-HHS-TIPS (800-447-8477)
Fax: 1-800-223-8164
Web: https://oig.hhs.gov/fraud/report-fraud/
Mail:
Office of Inspector General
Department of Health & Human Services
Attn: Hotline
P.O. Box 23489
Washington, DC 20026

USDA OIG Hotline contact information:

Voice: 1-800-424-9121
Fax: 1-202-690-2474
Web: https://usdaoig.oversight.gov/resources/hotline-information
Mail:
United States Department of Agriculture
Office of Inspector General
P.O. Box 23399
Washington, DC 20026-3399

12.5 Registration of Possession, Use or Transfer of Select Agents.

All activity involving Select Agents must be registered with the Reed Office of Environmental Health & Safety prior to initiation.

12.6 Discovery of Select Agents or Unknown Samples

Please notify EHS immediately if:

- You identify any Select Agent pathogen or toxin listed on the current federal list that was not previously registered by your lab
- You discover a toxin not previously reported by your laboratory in excess of the federal maximum allowable quantities listed above.
- You discover any unknown materials in your laboratory for assistance with identification.

These discoveries must be reported to the applicable governmental institution.

12.7 Intrafacility Transfer of Select Agents

Select agent pathogens and toxins may not be transferred outside of, to, or within Reed College unless EHS and federal approval has been granted. An intrafacility transfer is defined as the transfer of a Select Agent from one EHS and federally registered Select Agent lab to a similarly

registered laboratory. Select Agents may not be transferred to a laboratory that is not registered with EHS and the applicable governmental institution. Once approved, intrafacility transfers will be overseen by EHS. Please contact the EHS Office for additional information.

12.8 Destruction of Select Agents or Unknown Samples

Select Agent pathogens or toxins may not be destroyed until EHS and the applicable government institution has provided approval for the destruction. Once approval has been granted for the destruction of Select Agents, EHS will officially assume possession of the material and record its destruction. The governing institution will alert Reed if witnesses are required.

If you have any questions regarding the Federal Select Agent process, please don't hesitate to contact the EHS Office.

12.9 Additional Information

Program may be found at the following web sites:
Reed EHS: https://www.reed.edu/ehs/EHS_programs/
Centers for Disease Control and Prevention: https://www.selectagents.gov/
Notice of Exclusion for attenuated strains SAs: https://www.selectagents.gov/sat/exclusions/
United States Department of Agriculture: http://www.usda.gov/

13.0 Research Involving Recombinant and Synthetic Nucleic Acid Molecules

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines) are a set of research requirements for any institutions receiving funding from the NIH. As Reed receives NIH funding, the NIH Guidelines must be followed by every PI at the College, regardless of their source of funding, which is a central condition of the NIH funding agreement.

13.1 Principal Investigator Responsibilities Under the NIH Guidelines

Principal Investigators are responsible for full compliance with the NIH Guidelines. Core responsibilities include:

- Identifying if their research is subject to the NIH Guidelines
- Determining which section of the Guidelines are applicable to their work
- Proposing an appropriate biocontainment level (BSL) to ensure that the research is performed safely
- Obtaining authorization from the Reed College Institutional Biosafety Committee prior to starting any research that is subject to the NIH Guidelines or the IBC requirements.

Non-exempt recombinant DNA experiments, which must be registered and approved prior to initiation, are further defined on the IBC webpage. These include experiments that involve:

- The deliberately transfer a drug resistance trait to a microorganism
- Human gene transfer
- Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of <100 ug/kg body weight
- Human or animal pathogens as host-vector systems
- Cloning of DNA or RNA from certain pathogens
- Recombinant DNA work in whole animals or plants
- Large-scale DNA work with more than 10 liters of culture

You can review the Initial Classification Form and the Recombinant DNA Research Questionnaire (RDRQ) which is located in Appendix B and C of this Manual as well as on the IBC webpage at https://www.reed.edu/ibc. The following table from the NIH provides an overview of rDNA experiments and the level of review required for each category.

13.1.1 Level of Review for rDNA Experiments

Level of Review	Example of rDNA Experiment	NIH Guideline Section
IBC, RAC review and NIH Director Approval (RDRQ needed)	Experiments that compromise the control of disease agents in medicine through deliberate transfer or a drug resistance trait	III-A
IBC Approval and NIH review (RDRQ needed)	Deliberate formation of rDNA containing genes coding for a toxin with an LD50 < 100 ng/kg	III-B
IBC and IRB Approval and NIH review (RDRQ needed)	Introduction of rDNA into human subjects (Human gene transfer)	III-C
IBC Approval before initiation (RDRQ needed)	Wide range (rDNA exp's involving pathogens, defective vectors, animals, plants, large scale) (BL1 or 2)	III-D
IBC Notice at initiation (RDRQ needed)	Creating transgenic rodents, low risk rDNA Plant experiments (Typically BL1)	III-E
Exempt – notification to IBC only via Initial Classification Form	Those do not represent a significant risk to health or the environment (handle at BL1)	III-F

Acronyms:
IBC = Institutional Biosafety Committee
IRB = Institutional Review Board
NIH = National Institute of Health
RAC = Recombinant DNA Advisory Committee
RDRQ = Recombinant DNA Research Questionnaire

Principal Investigators are responsible for ensuring that their researchers are trained in safe work practices and that everyone working in the laboratory is aware of the emergency response procedures that must be followed after an incident. Staff must also be alerted of the rationale for any special medical surveillance restrictions or immunizations. Once work has been initiated, Principal Investigators must also supervise and monitor staff for their adherence to safety protocols.

If the scope of research changes significantly, the Principal Investigator must update their rDNA protocol with the Reed Institutional Biological Safety Committee and await confirmation in writing that the protocol change has been approved.

It is also important that Principal Investigators report any significant problem, such as a violation of the NIH Guidelines or any significant research related accidents, exposures or illnesses to the Reed College Environmental Health and Safety Office and the NIH Office of Biotechnology Activities.

14.0 Shipping

Shipments of research materials may be regulated by many regulatory agencies including:

- United States Department of Transportation (DOT)
- International Civil Aviation Organization (IACAO)
- International Air Transport Association (IATA)
- United States Department of Commerce (DoC) – Exports
- Centers for Disease Control and Prevention (CDC) – Imports
- United States Department of Agriculture (USDA) – Imports and transfers within the US

To facilitate compliance with the regulations, all hazardous materials shipments are managed by EHS and Mail Service. Anyone involved in the shipment of research materials must have detailed knowledge of the material being shipped. Because of this, administrative staff shall not process research material shipments.

14.1 Training

All hazardous materials shipments require shippers to be authorized to perform this task. EHS and Mail Services provides this service to the Reed Community. Please contact either EHS or Mail Services for assistance with hazardous materials shipments, including dry ice.

14.1.1 Table of Required Permits

Agency	Material to be Imported
CDC Call (404) 639-3883 or visit the CDC website at https://www.cdc.gov/orr/ipp/ for further information	**Etiologic agents** - infectious agent known to cause disease in man. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts and molds. In some instances, agents which are suspected of causing human disease also require a permit.
	Biological materials - Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected of being infected with disease transmissible to man.
	Animals - Any animal known or suspected of being infected with any disease transmissible to man. Importation of turtles of less than 4 inches in shell length and all non-human primates require an importation permit issued by the Division of Quarantine.
	Insects - Any living insect, or other living arthropod, known or suspected of being infected with any disease transmissible to man. Also, if alive, any fleas, flies, lice, mites, mosquitoes, or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.

<table>
<tr><td rowspan="2"></td><td>Snails - Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either Centers for Disease Control and Prevention or the Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.</td></tr>
<tr><td>Bats - All live bats. Bats may also require a permit from the U.S. Department of Interior, Fish and Wildlife Services.</td></tr>
<tr><td rowspan="7">USDA
Call (410) 436-8226 or visit the USDA website at https://www.aphis.usda.gov/aphis/resources/permits for further information</td><td>Materials derived from animals or exposed to animal-source materials. Materials which require a permit include:
• Animal tissues
• Blood
• Cells or cell lines of livestock or poultry origin
• RNA/DNA extracts
• Hormones
• Enzymes
• Monoclonal antibodies for IN VIVO use in non-human species
• Certain polyclonal antibodies
• Antisera
• Bulk shipments of test kit reagents,
• Microorganisms including bacteria, viruses, protozoa, and fungi.

Exceptions to this requirement are human and non-human primate tissues, serum, and blood.</td></tr>
<tr><td>Dairy products (except butter and cheese), and meat products (e.g., meat pies, prepared foods) from countries with livestock diseases exotic to the U.S.</td></tr>
<tr><td>Foreign plant pests injurious to plants grown in the United States.</td></tr>
<tr><td>Designated noxious weeds, which are of foreign origin and new to or not widely prevalent in the United States.</td></tr>
<tr><td>Insects, Mites, and Nematodes Introduced for biological control of weeds in the United States.</td></tr>
<tr><td>Domestic Plant Pests regulated by Federal or State Quarantines.</td></tr>
<tr><td>USFWS (U.S. Fish & Wildlife Service)</td><td>Certain live animals and all live bats.</td></tr>
</table>

Appendix A External Resources for Human Etiological Agent Classification and Evaluation according to Risk Groups and Biosafety Levels

A.I - NIH Guidelines and Risk Group Classification

Human Etiological Agents are sorted into Risk Groups based on an evaluation of the degree of hazard they pose to a healthy adult human. These Risk Groups include those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Mutated, recombined, and non-pathogenic species and strains are not considered, and non-infectious life cycle stages of parasites are also excluded.

Information on agent risk assessment may be found in the Agent Summary Statements of the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (see Sections V-C, V-D, V-E, and V-F, Footnotes and References of Sections I through IV.)

For further information on etiological agents, consult the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), which were last updated in April 2019 (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf). Appendix B of the NIH Guidelines contains a full list of known etiological agents and their risk group classifications.

For a table detailing the basis for classification of biohazardous agents, see below.

Appendix A - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

A.II - BMBL and Biosafety Levels

The CDC and NIH have established biosafety guidelines that are found in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), available here: https://www.cdc.gov/labs/BMBL.html.

Unlike the NIH guidelines, the BMBL guidelines are not regulatory; instead, they are intended to be used as an advisory tool for the establishment of assessment and proposed mitigation procedures in biomedical and clinical laboratories.

Because it is not possible for a single document to identify all of the possible combinations of risks and mitigations feasible in biomedical and clinical laboratories, there is not a precise correspondence between NIH Risk Groups and BMBL Biosafety Levels. Nevertheless, it is generally recommended that a laboratory adopt safety protocols following BMBL guidelines for a Biosafety level *at least equal* to the corresponding Risk Group of experimental materials (i.e for materials that are classified under Risk Group 2, it is recommended that Biosafety Level 2 protocols *or higher* be adopted).

Work may be conducted at a higher biosafety level than the Risk Group of the material if the institution and PI have identified additional risks associated with laboratory work (for example, an immunocompromised staff member), or at a lower Biosafety Level than the Risk Group of the corresponding material if the material has been subjected to treatments to lower risk (for example, virus that has been inactivated). The latter course of action is not recommended without extensive risk evaluation and approval from the Principle Investigator, the Institutional Biosafety Committee, and EHS.

Reed College is not currently authorized to conduct work at Biosafety Levels above 1 and 2. Additionally, Reed College laboratories are not currently equipped to be able to comply with safety guidelines at Biosafety Level 3 or higher. Although work practices at Biosafety Level 2+ are detailed in Section A.III below for possible use in case of an increased risk factor (for example, an immunocompromised laboratory staff member), any work with materials classified above Risk Group 2 and/or which would require a Biosafety Level greater than 2 would require pre-authorization from the IBC, IRB, and EHS; purchase of additional PPE, and possibly laboratory remodeling.

For additional information please contact EHS.

A.III - BL2+ Work Practices

Biosafety Level 2 Plus (BL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BL2 procedures. Generally, BL3 practices are mandated in a space designed for BL2 work. It is preferred that the BL2 laboratory be self-contained; that is, all equipment required for the experiment should be located within the lab. A sign is posted on the door while BL2+ work is in progress, and access is restricted to those involved in the experiment. When work is completed, and equipment has been decontaminated, the sign is removed and the lab returns to standard BL2 or BL1 usage.

BL3 practices require that all work be conducted under physical containment. Therefore, all manipulations with BL2+ material are conducted within a Class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures. The following notes further describe the requirements for work at BL2+.

Personal Protective Equipment (PPE)

- Dedicate PPE for the experiment. PPE worn for BL2+ work should not be worn in other areas. Remove before leaving the laboratory.
- Wear a lab coat or solid-front gown, preferably with a knit or grip cuff.
- Double glove for all work within the biological safety cabinet (BSC). Remove the outer pair before exiting the BSC, and don a new pair each time you reenter the BSC.
- Ensure that your gloves extend over the sleeve of your lab coat. An opening at the wrist will allow aerosols generated within the BSC to contaminate your wrist and forearm, extending hand-washing to your elbow.
- Sleeve covers can be worn to ensure coverage of the wrist and will also minimize contamination of the sleeves of your lab coat.
- Face Protection (mask and eyewear) can also be worn and will protect mucous membranes from exposure in the event of a spill outside the BSC during transfer of material to and from the incubator. It will also help to prevent you from touching your eyes, nose and mouth when working within the BSC.
- Remove PPE before leaving the laboratory. Placing a coat hook within the BL2+ area will facilitate this requirement. Remove your outer gloves first, then your lab coat or gown, followed by the inner gloves. Take your face protection off last. Don't touch your face with gloved hands. Remove gloves and other clothing aseptically, from the inside out, and avoid touching the contaminated outer side of the glove.
- Decontaminate reusable PPE as soon as feasible after it has been contaminated. Small areas can be spot treated with a suitable disinfectant, such as 1-10% household bleach. Lab coats can also be autoclaved, or sent to a laundry facility equipped to handle biohazardous PPE. Disposable PPE can be placed within a biohazard bag, treated and discarded as biomedical waste.
- Wash your hands with soap and water after removing PPE and before leaving the laboratory. Work Practices in the Biological Safety Cabinet (BSC)

- Perform all work within a BSC. This includes discarding waste within the BSC. Moving your hands in and out of the BSC will disrupt the protective air curtain at the front access opening.
- Place all items required for the experiment within the BSC before starting work.
- Wipe items down with disinfectant prior to placement within the BSC.
- Segregate clean areas from contaminated areas within the BSC (by at least 12-14").
- Keep the front and rear grilles clear when working within the BSC. Avoid blocking the rear grille. Don't store items on top of the BSC. Remind fellow researchers to minimize traffic and work behind the operator, as this may interfere with cabinet airflow. Depending on the location of the BSC within the room, opening and closing the room door can significantly interfere with BSC airflow.
- Avoid the use of a flame within the BSC. In addition to presenting a fire hazard, an open flame can disrupt airflow and possibly damage the paper filter located above the work surface. If the use of flame is absolutely necessary, use a burner with a pilot light that provides a flame only when depressed and releases after contact. Never leave an open flame (burner or pilot light) unattended in your BSC.
- Store tissue culture flasks in the incubator within small secondary trays to help minimize contamination. Trays will also facilitate transfer to and from the BSC.
- Keep your hands away from your face (face protection helps to minimize the potential for this route of exposure).
- Avoid the use of glass Pasteur pipettes or needles and syringes. Substitute plastic for glass whenever feasible. Alternatives to glass Pasteur pipettes include: plastic pipettes, plastic transfer pipettes, plastic gel loading pipette tips and pipette tip extenders, aspirators, and flexible plastic aspiration pipettes. Some researchers will either score and break the end off of a 1 ml or 5 ml plastic pipette or remove the wool plug and use it for aspirating cultures.
- If the use of sharps cannot be avoided, maintain a sharps container in the immediate vicinity of use. Discard intact needles and syringes immediately after use. Use a one-handed disposal method (keep a hand behind your back or by your side, don't place it on or near the opening of the sharps container). Never recap, bend, break or otherwise manipulate sharps by hand. If you must remove the needle from the syringe, use the small opening on the top of the needlebox for this purpose. Forceps, tweezers, or small pliers may also be utilized.
- Protect the house vacuum system or pump from contamination by installing a trap and filter system. Use a primary collection flask containing disinfectant, followed by an overflow flask, which leads through a HEPA or hydrophobic filter.
- Collect all waste within the BSC. Smaller biohazard waste bags may be utilized along with beakers or shallow trays containing disinfectant for the collection and disinfection of pipettes and other contaminated items. Waste can also be collected within the BSC in the following manner:
    - Horizontal collection: Horizontal trays containing disinfectant allow total immersion of pipettes.

    - Vertical collection: Beakers containing disinfectant can be used if disinfectant is drawn up inside the pipette and allowed to run down the interior wall upon disposal into the beaker.
    - Bags: Bags have the potential for creating aerosols when moved. At BL2+, seal autoclave bags within the cabinet and place within a second bag. Carefully add water to the primary bag before sealing (25 ml for smaller bags, 200 ml for larger bags). The addition of water will help to generate steam within the bag during the autoclave cycle.
- Wipe items down with disinfectant prior to removal from the BSC.
- Wipe down BSC with disinfectant after use (work surface, grilles, sides, back and inside front view screen).
- Decontaminate liquid waste with household bleach diluted 10% against the volume of the waste. Allow at least a 30-minute contact time for full decontamination.
- Transport waste to autoclave in a leak-proof container.

Centrifugation

- Use sealed rotors or safety buckets as secondary containment for centrifugation.
- Load and unload the rotor or safety buckets within the BSC.
- Don't overfill primary containers, limit to < ¾ full. Wipe exterior of tube with disinfectant before loading.
- Seal rotor or bucket and wipe down with disinfectant, remove outer gloves, and transport to the centrifuge.
- Post a sign on centrifuge that includes the biohazard symbol, name of the agent with Biosafety Level, and your name.
- Wait 2-5 minutes after the run to allow aerosols to settle in the event of a spill. Transport sealed rotor or safety bucket to BSC to complete your experiment. Don new pair of outer gloves.
- Decontaminate the rotor or safety bucket by spraying with 70% ethanol and allowing to air dry. Wipe the throw line within the centrifuge with disinfectant and remove your biohazard sign. In the event of a spill during centrifugation, follow the spill response procedures.
- Avoid the use of microfuge, which is difficult to contain. If you cannot avoid using a microfuge, use a model that has built in secondary containment (a sealed rotor) along with microfuge tubes equipped with an O-ring seal. You can also operate your microfuge in the rear of your BSC (don't perform any work within the BSC while the microfuge is in operation, and wait 2-5 minutes after the run before opening the microfuge).

Labels

- Post a biohazard sign at the entry to the BL2+ laboratory.
- Ensure that any specific entry requirements (vaccination), the name of the agent, the Biosafety Level, and the name of an emergency contact person ARE posted on the sign.

- Label equipment housing the agent (incubators, freezers) with the universal biohazard symbol and agent name.

Transport of Biohazards on Campus (between labs or buildings):

Must have two leak-proof containers, including the following:

- a sealed primary container
- a sealed secondary container
- absorbent (paper towels) between the primary and secondary containers suitable for the volume transported
- a biohazard sticker on the outside of the secondary container with agent name
- lab address and phone number on the outside of the secondary container

Utilize plastic containers whenever feasible; avoid glass. Sealed plastic (not glass) primary vials can be transported within sealed, labeled plastic bags. If glass primary containers must be used, place containers within a sealed rigid plastic container with absorbent and padding to cushion vials during transport.

Decontaminate the outside of the primary container before placing it into the secondary container. Decontaminate the secondary container before leaving the laboratory.

Hand-washing

- Wash hands whenever PPE is removed and before leaving the laboratory.
- Wash with soap and warm water for at least 15 seconds. Since the contact time of most soaps is quite extensive for actual decontamination, mechanical friction from scrubbing and water dilution are essential for complete cleaning.
- No glove is 100% leak-proof.
- Never wet or hand wash your gloves with water or disinfectant, as this will encourage wicking and increase permeability of the protective barrier.

Spills and Exposure Incidents

All researchers must be familiar with the applicable exposure response procedures before initiating their experiment

Appendix B Initial Classification Form

REED COLLEGE

Initial Classification Form for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

This information is required to determine if your research is exempt from IBC oversight based on Federal requirements (see NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules). If your research is exempt, you will receive notification from the IBC. Otherwise you will be required to complete an additional form for IBC review. For more information please see IBC website: reed.edu/ibc

Submit the completed form to ibc@reed.edu

Principal Investigator (Last Name, First Name, Degree)

_____

Email Address _____ Telephone Number _____

Lab location (bldg., rm) _____ Laboratory Contact (optional) _____

Project Title _____ Project Sponsor

_____

Please check all that apply to your research project and <u>teaching lab activities</u>:

- ☐ This research involves transformation of standard laboratory strains of bacteria or yeast (e.g., *Escherichia Coli K-12*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, or any asporogenic *Bacillus subtilis* or *Bacillus licheniformis*) with standard plasmids (see Appendix C of NIH Guidelines).
- ☐ This research uses a host-vector system that is **NOT** *Escherichia Coli K-12*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, or any asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C of NIH Guidelines). *If checked, please list system(s) used:*
- ☐ This research involves transfection of tissue culture cells with non-viral vectors (e.g., plasmids).
- ☐ This research uses viral vectors or any vector containing >50% of the genome of a eukaryotic virus.
- ☐ This research involves the use of synthetic nucleic acid molecules in cells, organisms or viruses. If checked, please check all that apply below:
    - ☐ The synthetic nucleic acid molecule can replicate or generate nucleic acids that can replicate.
    - ☐ The synthetic nucleic acid molecule can integrate into host DNA.
- ☐ This research involves transgenic/knockout rodents.

- ☐ This research involves the creation of other transgenic animals (e.g., flies, fish), including the generation of new strains by breeding two different strains.
- ☐ This research involves the use of recombinant plasmids/vectors/viruses, including recombinantly modified cells/organisms, in animals (other than the creation of transgenic animals as noted above).
- ☐ This research involves the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, and acquisition could compromise the use of a drug used to control disease in humans, veterinary medicine, or agriculture (see Section III-A-1-a of NIH Guidelines).
- ☐ This research involves the deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at a LD50 of less than 100 ng/kg body weight (see Appendix F of NIH Guidelines).
- ☐ This research involves DNA from or synthetic nucleic acid molecules encoding a significant human pathogen (defined as risk group 3 or 4) or cells known to be infected with these agents (see Appendix B of the NIH Guidelines). *If in doubt, list proposed agent:*
- ☐ This research involves more than 10 liters of culture in a single container.
- ☐ Other experiments involving recombinant or synthetic nucleic acid molecules are proposed that are not covered here. *If checked, please explain:*

Assurances

- ☐ *I will abide by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and the CDC Guidelines for Biosafety in Microbiological and Biomedical Laboratories.*
- ☐ *I will maintain a current record of any transfer of recombinant or synthetic nucleic acid molecules, or vectors or host strains containing recombinant or synthetic nucleic acid molecules, infectious agents or biological toxins between investigators at this or other institutions.*
- ☐ *I agree that as principal investigator it is my responsibility to make certain that prior to engaging in research involving known or potential pathogens, all laboratory and support personnel are properly trained in the practices and techniques required to ensure safety, and to supervise the safety performance of those involved ensuring that the required safety practices and techniques are employed.*
- ☐ *I agree to send a revised project description to the Institutional Biosafety Committee if changes are made to portions of this project involving recombinant or synthetic nucleic acids and will not begin work prior to IBC approval.*

Principal Investigator Signature Date

Head of lab (if different) Signature Date Print name (head of lab)

Appendix C Recombinant DNA Research Questionnaire (RDRQ)

REED COLLEGE

Recombinant DNA (rDNA) Research Questionnaire (RDRQ) for Exempt and

NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules

(Please answer questions completely — Use additional space as necessary)

Submit form and all supporting documents to ibc@reed.edu

IBC #	Original Submit Date		☐ **Modification**	☐ **Five Year Renewal**
	☐ **Annual Review Year 1 Date:**	☐ **Annual Review Year 2 Date:**	☐ **Annual Review Year 3 Date:**	☐ **Annual Review Year 4 Date:**

Project Title: _____

Department: _____

Telephone: _____ Email: _____

List all staff/faculty/students that will be working on project:

_____

_____

Brief Project Summary (referencing the relevant section(s) of the NIH Guidelines). Include all work within your research laboratories as well as within your teaching laboratories.

Please answer all questions on this form. You may indicate "N/A" if a particular question is not applicable.

1. Please provide overview of rDNA molecules to be used, and whether *in vivo* uses are proposed *(use additional rows as necessary)*

Vector type/Agent	Insert(s) or Deletions	Host (cell type and/or species)
Example: lentivirus *Example L. pneumophila*	*GFP* *LP01*	*Mice (in vivo)* *Human fibroblasts (in vitro)*

Vector/Agent Detail

2. Vectors to be used (plasmids, cosmids, phages, viruses) — specify type and strain, and give description:

   a. If plasmids are used in the packaging system for the viral vector, they must also be listed and described.
   b. If a packaging cell line is used for the viral vector, it must also be listed.

In addition, please attach commercial product literature, a vector map, a copy of any journal articles describing construction of vector or provide a web link to specific information within this document. Also state whether you will be producing the vectors, or obtaining them commercially.

3. For viral vectors, describe how the vector differs from the original virus (in terms of pathogenicity and genome size): *[e.g., E1 & E3 genes deleted from adenoviral vector]*

4. For recombinant infectious agents, describe how it differs from the wild type infectious agent (in terms of pathogenicity, virulence, or immunogenicity): [e.g., attenuated Listeria Monocytogenes]

5. List all drug resistance genes that are NOT for the purpose of plasmid propagation in *E.coli* or for plasmid transfection in *in vitro* cell culture. Could any of these drug resistance genes compromise the use of the drug to control disease caused by this agent in humans, animals, or agriculture?

6. Will infectious virus particles or other infectious agents, either replication-deficient or replication competent, be rescued, propagated or purified in your laboratory?

7. What is the host range of the viral vector or recombinant infectious agent?

8. Will there be surveillance for production of wild type or replication competent infectious agents? Discuss.

DNA Insert(s) and Expression

9. State DNA Source (species, tissue/cell, or microbiological agents).

10. What gene products will be expressed or knocked down (shRNA or gene deletion) and what is the functional impact of overexpression or knockdown? If the gene product being expressed is from a Commerce Control Agent please provide citations describing the function of the gene product.

11. Please describe potential human, animal, or plant health hazards of the gene product or its transcribed or translated products (e.g., could it be oncogenic or toxic?). Please provide the vertebrate LD50 for any genes encoding a biological toxin.

12. Would any of the gene inserts or deletions potentially increase the virulence of the recombinant virus or recombinant pathogenic organism or allow the infectious agent to evade the host immune response? If yes, discuss.

13. Will any gene be intentionally mutated? If yes, describe.

Host (cell type and/or species)

14. For in vitro experiments list host cells to be targeted (bacterial, eukaryotic, species).

15. If transduced or infected cells will be used for downstream applications (eg FACs sorting or in vivo transplant) when will these experiments occur relative to the time of transduction or Infection?

16. Are viral sequences present in the host that could recombine with the vector and lead to replication-competency for the recombinant construct, or has the host been previously infected with another agent?

17. For in vivo experiments describe the method of delivery, including a range of doses and numbers of administrations for each animal/agent. Please list registered IACUC protocols.

18. For whole animals, could there be an adverse physiological impact? Discuss.

19. For in vivo experiments, specifically discuss the potential for shedding of the agent from the animal host.

20. For in vivo experiments, will fluids, tissues or cells be harvested from the animal? Briefly describe harvested material and discuss if the material is potentially infectious or may contain replication competent infectious material.

Biosafety

21. Discuss the biohazard implications including potential exposure to staff and animal colonies. Describe protective clothing/equipment that personnel are required to wear/use. Include other preventative measures (e.g., vaccinations) if applicable.

22. Describe consequences of and response to accidental exposure, e.g., spill, mucosal splash, inhalation, or inoculation, which might occur during experimental handling.

23. Describe additional locations where these experiments (both in vitro and in vivo) may take place.

24. Describe equipment to be used (e.g., flow cytometer, centrifuge, microscope, animal imaging), or transportation to other locations that may require additional biosafety precautions.

25. Describe how materials containing rDNA or infectious agents (viral or other gene expression vectors, transfected cell lines, infected tissues, etc.) will be disposed/discarded.

26. Describe any additional concerns or proposed modifications to standard biosafety protocol.

Agreements

☐ I will abide by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, the CDC Guidelines for Biosafety in Microbiological and Biomedical Laboratories, and Reed College policies and procedures for research involving rDNA and/or synthetic nucleic acid molecules. (For reference, these are: https://osp.od.nih.gov/biotechnology/nih-guidelines/ http://www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm)

☐ I will maintain a current record of any transfer of recombinant DNA and/or synthetic nucleic acid molecules, or vectors or host strains containing recombinant DNA and/or synthetic nucleic acid molecules, or infectious agents between investigators at this or other institutions.

☐ I will contact EHS or mail services before shipping any hazardous materials.

☐ I will follow DOT, IATA, and CITES requirements and will ensure any laboratory personnel have received the required training, when applicable, for shipment of biological materials.

☐ I agree that as principal investigator it is my responsibility to make certain that prior to engaging in research involving known or potential pathogens, all laboratory and support personnel are properly trained in the practices and techniques required to ensure safety, and to supervise the safety performance of those involved ensuring that the required safety practices and techniques are employed.

☐ I agree to send all project modifications to the Institutional Biosafety Committee if changes are made to the recombinant DNA and/or synthetic nucleic acid molecule experiments described in this questionnaire.

If you have an electronic signature, please sign below, save, and return via email to ibc@reed.edu. If you do not have an electronic signature, please print this document, and sign the hard copy below.

Principal Investigator Signature Date

Head of Lab (if different) Signature Date

Biosafety Committee/BSO Approval Date

Appendix D Sources of Contamination

If contamination is experienced in the laboratory, the following items may be sources of the contamination. For additional assistance please contact EHS.

- Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., do not belong in the laboratory. These articles should be stored elsewhere.
- Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens or supplies, and outside air is a complication that may affect or invalidate the results of an experiment. Human sources of this type of contamination are evaluated as follows:
    - Sneezing, coughing and talking.
        - Sneezing, variously reported to generate as many as 32,000 or 1,000,000 droplets below 100 microns in diameter;
        - Coughing, which produces fewer and larger droplets; and
        - Talking, which has been reported to average only 250 droplets when speaking 100 words, show great differences between persons in regard to the number of microorganisms aerosolized.

      As a general rule, it may be said that these actions by normal healthy persons may play a less important role in transmission of airborne infection to humans or experimental materials than does liberation of microorganisms from human skin.
    - Dispersal of bacteria from human skin.
        - There is a tremendous variation in the number of bacteria shed from the skin by a clothed subject.
            - For instance, in one study, the number varied from 6,000 to 60,000 per minute. These bacteria were released on skin scales of a size that could penetrate the coarse fabric used for the laboratory and surgical clothing in the test. Dispersal of skin bacteria was several times greater from the area below the waist than from upper parts of the body.
        - Effective reduction is accomplished by use of closely-woven or impervious clothing fitted tightly at the neck, wrists, and ankles to prevent the clothing from acting as bellows that disperses air carrying skin scales laden with bacteria. Such clothing sometimes is too warm to work in.
    - Prolific dispersal of bacteria occurs from infected abrasions, small pustules, boils, and skin disease.
        - Washing of lesions with germicidal soap will greatly decrease the number of organisms on the skin and dispersal into the air.

        - Healthy nasal carriers who generate aerosolized staphylococci usually can be identified by the presence of heavy contamination of their fingers, face, and hair. This point may be useful in investigating the source of staphylococcal contamination of cell lines.
    - Footwear. In moderate and high-risk situations, shoes reserved only for laboratory use have been recommended as a precaution against transporting spilled infectious agents outside the laboratory. In experiments during which reduction of potential contamination of experimental materials is important, laboratory-only shoes can also reduce the microbial load brought into the laboratory each day by street shoes. Shoes are efficient transporters. In one study, there were 4 to 850 times as many bacteria per square centimeter on the laboratory footwear as on the floor itself.

Personal Work Practices

Food, candy, gum, and beverages for human consumption will be stored and consumed only outside the laboratory. Smoking is not permitted in the laboratory or Reed College buildings. Shaving and brushing of teeth are not permitted in the laboratory. Razors, toothbrushes, toiletry supplies and cosmetics are permissible only in clean areas, and should never be used until after showering or thorough washing of the face and hands.

A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination, because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate facial fit of a face mask or respirator when the work requires respiratory protection.

Develop the habit of keeping hands away from mouth, nose, eyes, face and hair. This may prevent self-inoculation.

For product protection, a person with long hair should wear a suitable hair net or head cover that can be decontaminated. This has long been a requirement in hospital operating rooms and in facilities where biological pharmaceutical products are manufactured. A head cover also will protect the hair from fluids, splashes, from swinging into Bunsen burner flames and Petri dishes, as well as reduce facial contamination caused by habitual repetitive manual adjustment of the hair.

Long flowing hair and loose flapping clothing are dangerous in the presence of open flame or moving machinery.

Rings and wristwatches also are a mechanical hazard during operation of some types of machines.

Contact lenses do not provide eye protection. Contact lenses must not be worn by persons exposed to caustic chemicals unless safety glasses with side shields, goggles or full-face shield are worn to provide full protection.

Plants, cut flowers, an aquarium, and pets of any kind are undesirable sources of yeast, molds and other potential microbial contaminants of biological experimental materials.

When employees are subject to potential occupational infection, the shower and/or face/hand-washing facilities should be provided with germicidal soap.

Personal cloth handkerchiefs should not be used in the laboratory. Disposable cleansing tissues should be available for use instead.

Hand washing for personal protection:

- This should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, or entry at the wrist.
- Throughout the day, at intervals dictated by the nature of the work, the hands should be washed. Presence of a wristwatch discourages adequate washing of the wrist.
- Hands should be washed after removing soiled protective clothing, before leaving the laboratory area, before eating and smoking.
- A disinfectant wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin.

Anyone with a fresh or healing cut, abrasion, or skin lesion should not work with infectious materials unless the injured area is completely protected, such as with waterproof bandages and double gloving.

Use of surgeon’s mask of gauze or filter paper is of little value for personal respiratory protection. It is designed to prevent escape of droplets from the nose or mouth. If use of biohazards demands respiratory protection, contact EHS for assistance.

Appendix E Biomedical Waste

E.1 Introduction

You play an important role in Reed's biomedical waste program if you generate waste in a laboratory. This guide will help you dispose of your biomedical waste in an easy and legal manner.

Our program is designed to protect the people who handle, transport and dispose of your waste. The program is also designed to protect the environment and minimize Reed's regulatory liability.

Remember: Radioactive or hazardous chemical wastes shall be disposed of through the radioactive waste stream or the hazardous chemical waste stream respectively.

Please note: Clean broken or unbroken graduate cylinders, Erlenmeyer flasks, and beakers can be disposed of through the general trash. Place the items in a cardboard box, seal it, and label it "broken glass".

E.2 Biomedical Waste Management

All biomedical waste must be contained in a sealed red sharps container, or red biohazard bag.

You can minimize costs by filling these containers efficiently and following the instructions in this guide.

Autoclaved containers and bags should be allowed to cool before being placed in a box.

Place all sharps containers and bags upright in the box. This will help minimize leaks and spills during transport.

When the box is full, seal the box and apply a contact label to the outside of the box signing off that the waste is packaged appropriately.

E.3 Definition of Biomedical Waste

Medical wastes includes the following categories:

Waste Cultures and Stocks of Microorganisms or Etiologic Agents, including:

- Cultures and stocks of infectious agents or microorganisms from facilities assigned to Biosafety Levels 1 and 2 (BL1, BL2).
- Cultures of specimens from pathological laboratories.

- Disposable containers, materials, and supplies that may have been contaminated during the manipulation of microbial cultures and stocks
- Wastes from the production of biologicals (including all tissue culture materials.)
- Live and attenuated vaccines.

Waste Human Blood, Blood Products, and Their Containers

- Waste human blood and blood products (e.g. blood plasma, platelets, red or white corpuscles, and other derived licensed products such as interferon, etc.)
- Items saturated or dripping with human blood or blood products.
- Items caked with dried human blood or blood products.
- Intravenous bags.

Used Sharps Waste

- used hypodermic needles and syringes (with or without the attached needles)
- pasteur pipettes
- disposable plastic pipettes
- scalpel blades
- razor blades
- blood vials
- test tubes
- needles with attached tubing
- broken plastic culture dishes
- unbroken glass culture dishes
- other types of broken and unbroken glassware that were in contact with infectious material, including microscope slides and coverslips.

Unused Sharps Waste

- Unused hypodermic needles, suture needles, syringes, and scalpel blades.

Waste Animal Carcasses, Body Parts, and Bedding

- Animal wastes purposely infected or known to have been exposed to Class 1 or 2 agents shall be autoclaved.
- Uninfected or "clean" animals shall be wrapped in a nondescript plastic bag and placed in the hazardous waste room refrigerator.

E.4 Look-Alike Waste

Look-alike waste is not considered medical waste. Look-alike waste is plastic or glass labware, lab matting and gloves that have not been in contact with infectious material. Look-alike waste is disposed of through a separate waste stream and should not be placed in the medical waste stream.

Items should be discarded in a manner to prevent physical injury to those people handling the waste. Glass and items that are capable of puncturing bags should be placed in a plastic lined cardboard box. Do not autoclave or chemically decontaminate look-alike waste.

All intravascular sharps are considered biomedical waste regardless of the presence of infectious material and must be discarded in sharps containers. Do not discard intravascular sharps in the look-alike waste stream.

E.5 Disposal Procedures

Sanitary Sewer

The sanitary sewer was designed for the disposal of certain liquid wastes. Use of the sanitary sewer reduces the chance for leaks or spills during transport and reduces disposal costs.

- Waste microbiological liquid stocks (Class 1,2 and 3 agents) shall be autoclaved or chemically disinfected and poured down the drain whenever possible.
- Human blood and body fluids do not need to be disinfected before being poured down the drain.
- Remember to rinse the sink area afterward. Disinfect if necessary.

Sharps Containers

Discard all intravascular sharps waste such as hypodermic needles, syringes (with/without the attached needles), scalpel blades, and suture needles in your red container. You may also deposit any other type of sharps waste into this container.

Red Biohazard Bags

Place small volume pathological waste, empty intact plastic liquid waste containers (with a residual volume of less than 20 cubic centimeters); intact plastic blood containers (with a residual volume of less than 20 cubic centimeters); intact plastic disposable containers; all other non-sharp materials and supplies that may have been contaminated during the manipulation of microbial cultures and stocks; and non-sharp waste from the production of biologicals (including tissue culture materials) in the bag.

Bags can be placed into a bioharzardous cardboard box for off-site incineration. Bags that have been autoclaved do not need to be placed in biohazard cardboard boxes for incineration, and can be put into the normal trash once the hazard warning labels are removed.